FILE 'EMBASE, SCISEARCH, BIOSIS, MEDLINE, USPATFULL' ENTERED AT 14:28:25 ON 28 FEB 2001

L1 54031 S (SEPARAT? OR ISOLAT?) AND (CAPTUR? OR AGGLUTINAT? OR ELISA OR
L2 42332 S (?ACRYLAMIDE? OR ?METHACRYLAMIDE? OR CROSS-LINK?) (5A) POLYME
L3 5694 S CARBOXYL? (5A) (NITROGEN OR (ITACONIC ACID?) OR (MALEIC ANHYD
L4 754 S L2 AND L3
L5 37 S L1 AND L4
L6 37 DUP REM L5 (0 DUPLICATES REMOVED)
L7 291 S (TRANSITION METAL?) AND L1
L8 1 S L4 AND L7
L9 55 S L7 AND L2
L10 55 DUP REM L9 (0 DUPLICATES REMOVED)
L11 901 S L1 AND L2
L12 37 S L11 AND L4
L13 37 S L11 AND L3
L14 1 S L13 AND L7
L15 37 S L1 AND L2 AND L3
L16 1 S L15 AND (TRANSITION? METAL?)
L17 76 S (?ACRYLAMIDE?) AND (TRANSITION METAL?) AND (CAPTUR?) AND (?AS
L18 57 S L17 AND (ISOLAT?)
L19 57 DUP REM L18 (0 DUPLICATES REMOVED)
L20 3851 S (TARGET? (3A) ISOLAT?)
L21 0 S L4 AND L20
L22 87 S L2 AND L20
L23 6 S L22 AND L7
L24 6 DUP REM L23 (0 DUPLICATES REMOVED)
L25 87 S L20 AND L2
L26 0 S L25 AND L3
L27 0 S L25 AND L3
L28 12 S L25 AND (TRANSITION METAL?)
L29 12 DUP REM L28 (0 DUPLICATES REMOVED)

L8 ANSWER 1 OF 1 USPATFULL

ACCESSION NUMBER:	2000:105722 USPATFULL
TITLE:	Capillary assays involving **separation** of free and bound species
INVENTOR(S):	Ullman, Edwin F., Atherton, CA, United States Stauber, Gregory B., San Ramon, CA, United States
PATENT ASSIGNEE(S):	ACLARA Biosciences, Inc., Mountain View, CA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6103537	20000815
APPLICATION INFO.:	US 1998-163717	19980930 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-60857	19971002 (60)

DOCUMENT TYPE:	Utility
PRIMARY EXAMINER:	Chin, Christopher L.
ASSISTANT EXAMINER:	Do, Pensee
LEGAL REPRESENTATIVE:	Rowland, Bertram I.Rae-Venter Law Group, P.C.
NUMBER OF CLAIMS:	10
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	2 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT:	2255

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention concerns methods for masking inhomogeneity of a member of a specific binding pair (sbp) employed in a capillary electroseparation. The method comprises binding the sbp member to synthetic particles that become localized during capillary electroseparation. Also disclosed is one embodiment of the present invention, which is a method for conducting a capillary electroseparation specific binding assay. The method involves the electroseparation of a labeled first member of a specific binding pair that is bound in a complex from labeled first member that is not bound in the complex. The complex comprises the first member and a second member of a specific binding pair. A combination is provided comprising a sample suspected of containing an analyte, a labeled first member of a specific binding pair, and a second member of a specific binding pair under conditions for forming a complex between labeled first member and the second member. The second member either initially or subsequent to the formation of the complex being covalently or noncovalently bound to synthetic particles that migrate uniformly during electroseparation. The combination is subjected to electroseparation and a determination is made as to whether the complex is formed. Also disclosed are kits for conducting a capillary electroseparation specific binding assay.

TI Capillary assays involving **separation** of free and bound species

SUMM This invention relates to the field of specific binding **assays** and in particular to **assays** involving the **separation** of free and bound species. The ability to measure quantitatively or to identify a wide variety of physiologically active compounds,. . . has become of increasing importance, both as an adjunct to diagnosis, drug discovery and therapy. For the most part diagnostic **assays** of physiological fluids or biological samples for one or more analytes have

required clinical laboratory determinations although there has been an increasing focus on being able to carry out **assay** determinations in the doctor's office and in the home. Numerous systems have been developed in efforts to try to address. . .

SUMM One problem arises with analytes whose presence in biological samples is relatively low. For these analytes greater **assay** sensitivity is necessary and, consequently, there is a continuing interest in providing improved and alternative methods to those which are. . .

SUMM Numerous labels are used in specific binding **assays** including, for example, enzymes. Enzyme specific binding **assays** comprise qualitative and quantitative procedures in which a specific binding reaction such as, in immunological cases, an antigen-antibody reaction, is monitored by enzyme activity measurements. The term **ELISA** is generally used for enzyme **immunoassays** (EIA) that require a **separation** step. Reagent excess **assays** of specific antibodies or antigens that use an enzyme label are sometimes called immunoenzymometric **assays**. There are two basic types of EIA's: heterogeneous (**separation** required) and homogeneous (**separation** free) **assays**. In the heterogeneous systems, the activity of the enzyme label is not affected by the antigen-antibody reaction and it must be **separated** into fractions, one being enzyme reagent bound to antibody (or a complex) and the other being free, unbound enzyme reagent,. . .

SUMM In the homogeneous systems, the enzyme activity of the **assay** solution is measured without a prior **separation** of the antibody-bound enzyme label from the free, unbound one, primarily because the activity of the bound enzyme label is. . . from the unbound one. The various heterogeneous and homogeneous EIA's can be further characterized as either competitive or non-competitive (immunoenzymometric) **assays**. The characterization depends on whether the unlabeled antigen and the antigen linked to an enzyme compete for a limited number. . . measured is allowed to react alone with an excess of immune reactant. For a more detailed discussion of various enzyme **assay** techniques, see "Enzyme **Immunoassay**" by Edward T. Maggio, CRC Press, Inc., Boca Raton, Fla., 1980. See also, for example, U.S. Pat. Nos. 3,690,834; 3,791,932;. . .

SUMM Sandwich **assays**, particularly sandwich **immunoassays**, are one form of non-competitive **assay** that has been employed to achieve higher sensitivity in relation to competitive **immunoassays**. **Immunoassays** generally involve the use of antibodies, both monoclonal and polyclonal. However, antibodies, even monoclonal antibodies, are relatively inhomogeneous. Additionally, it. . . Accordingly, labeling of antibodies tends to further increase heterogeneity of antibody reagents. Other types of binding substances used in binding **assays** can be even more heterogeneous. For example receptors, particularly membrane bound receptors are frequently **isolatable** only when bound to other highly heterogeneous components.

SUMM Electrophoresis has been used for the **separation** and analysis of mixtures. Electrophoresis involves the migration and **separation** of molecules in an electric field based on differences in mobility. Various forms of electrophoresis are known including free zone electrophoresis, gel electrophoresis, isoelectric focusing and isotachophoresis. One approach to **immunoassays** employs capillary electrophoresis for **separation** of free and bound label. In capillary electrophoresis electroseparation is performed in tubes or channels of micrometer cross-sectional dimensions. Capillary electrophoresis may be used to **separate** an antibody-antigen complex from either the unbound form of the antigen or the antibody.

Either the bound or free species. . .

SUMM Although sandwich specific binding **assays** can provide much higher sensitivity than competitive **assays**, the heterogeneity of labeled receptors and antibodies makes the capillary electroseparation difficult to carry out. This results because the unbound. . . than sharp, well defined, distributions upon electroseparation analysis. Thus, conventional electroseparation methods may not offer significant advantages for specific binding **assay** applications.

SUMM . . . describe a method for simultaneous quantification of multiple drug analytes in urine, based on combining immunochemical binding with capillary electrophoretic **separation** and laser-induced fluorescence.

DETD . . . device or a spinner flask device, both of which are well known in the art. Various conventional ways exist for **isolation** and purification of the monoclonal antibodies from other proteins and other contaminants (see Kbhler and Milstein, supra).

DETD . . . such as alkaline phosphatase and so forth, a chemiluminescent compound such as luminol, acridinium compound, electroluminescent group such as a **transition metal** complex (see, e.g., U.S. Pat. Nos. 5,541,113, 5,610,017, 5,527,710, 5,591,581, the relevant disclosures of which are incorporated herein by reference),. . .

DETD . . . a member of a specific binding pair that manifests itself by the non-uniform migration of the sbp member in a **separation** process, such as electroseparation. The inhomogeneity may arise in an sbp member because of heterogeneity resulting from the presence of. . .

DETD . . . is significantly reduced or eliminated thereby resulting in a sharp, well-defined band of the sbp member during migration in a **separation** process such as electroseparation.

DETD Electroseparation--**separation** of components in a liquid by application of an electric field, preferably, by electrophoresis (electrokinetic flow) or electroosmotic flow. Various. . .

DETD . . . magnetic fields, and the like. Again, the idea of the present invention is to achieve sufficient localization such that a **separation** of bound and free species can be achieved.

DETD . . . However, the particles may be charged but not in the sense that the charge is the primary factor in the **separation** of the free and bound species.

DETD . . . such as agarose, which is available as Sepharose, dextran, available as Sephadex and Sephacryl, cellulose, starch and the like addition **polymers** such as polystyrene, **polyacrylamide**, homopolymers and copolymers of derivatives of acrylate and methacrylate, particularly esters and amides having free hydroxyl functionalities. Metal sols include. . .

DETD . . . to amine groups, carboxyl groups, active olefins, alkylating agents, e.g., bromoacetyl, to form the linking member. Where an amine and **carboxylic** acid or its **nitrogen** derivative or phosphoric acid are linked, amides, amidines and phosphoramides will be formed. Where mercaptan and activated olefin are linked,. . .

DETD The paramagnetic particles should be readily suspendable and form stable, preferably colloidal, suspensions and should **separate** in a few seconds in a capillary subjected to a magnetic field strength of 1 to 5 Kgauss. Exemplary of. . .

DETD . . . medium is sufficient to permit the migration of the sbp member-paramagnetic particle conjugate in a sharp, well-defined band to allow **separation** of free and bound species. The strength of the magnetic field is usually about 0.1 to 10 Kgauss, preferably, about. . .

DETD Sieving gel--sieving gels are commercially available gels with defined pore sizes for **separating** different size solutes. Examples of suitable sieving gel components are, by way of illustration and not limitation, agarose, ficoll, polysucrose,. . .

DETD **Assay**--a method for determining a substance capable of binding to a specific binding pair member, for example, for determining an analyte. . . . or detecting the degree of binding of a compound to a receptor. The determination may be qualitative or quantitative. Such **assays** depend on specific binding of a ligand to its receptor and include receptor binding **assays**, **immunoassays**, ligand/binding **assays**, polynucleotide **assays**, particularly polynucleotide hybridization **assays**, and cell surface binding **assays**. The **assays** may be utilized for drug discovery and screening, studies of receptors, detection of drugs and other substances, DNA detection, DNA sequencing, genetic analysis, monitoring of gene expression, and so forth. One particular **assay** is the **immunoassay**, which is a specific binding **assay** in which the reagents include an antibody.

DETD Heterogeneous **assay**--an **assay** wherein free labeled species is **separated** from a labeled species that is bound to another species such as an sbp member. The **separation** may be carried out by physical **separation**, e.g., by transferring one of the species to another reaction vessel, and so forth, and may include one or more washing steps. The **separation** may be nonphysical in that no transfer of one or both of the species is conducted, but the species are **separated** from one another in situ such as by differences in size, differences in mobility, differences in charge to mass ratio and the like. Nonphysical **separation** techniques are more applicable to the area of microfluidics. In addition, magnetic beads may be employed for **capture** and **separation**. In the heterogeneous **assay** the activity of a label is not affected by the reaction of specific binding pair members with one another. Regardless of the means of **separation**, the signal from the label may be measured from one or both of the **separated** species.

DETD Homogeneous **assay**--an **assay** wherein free labeled species is not **separated** from a labeled species that is bound to another species such as an sbp member. The signal from the label is significantly different between the free labeled species and that which is bound and, thus, can be measured without **separation**.

DETD **Immunoassay**--a specific binding **assay** in which the reagents include an antibody.

DETD . . . that become negatively charged. Alpha-diketones are subjected to electrochemical reduction when in proximity of an anode in contact with the **assay** medium.

DETD . . . nature of the synthetic particles employed determines the manner in which the particles are treated during the electroseparation to achieve **separation** from free labeled sbp members. Synthetic particles that have uniform dimensions and charge, generally can be caused to migrate uniformly. . .

DETD . . . where a sieving gel is not used. In general, the higher the ionic strength of the medium, the worse the **separation** between the particle species. The ionic strength of the medium is usually about 0.005 to 0.6 mM, more typically, about 0.02 to 0.10 mM. The length of the **separation** path is also a consideration. In general, the longer the length of **separation** path, the worse the **separation**. The **separation** path is usually about 1 mm to 20 cm, more typically, about 2 mm to 5 cm. The length of the **separation** path varies depending on the particular species. Thus, for example, for single base DNA **separation** a typical **separation** length is about 4 cm.

DETD . . . above parameters for the electroseparation including those for the medium and the electric potential are usually optimized to achieve maximum **separation** of free and bound species. This may be achieved empirically and is well within the purview of the skilled artisan.

DETD . . . the second sbp member. Generally, it is preferable for the binder to be attached to a particular polymer that facilitates **separation**. However, in some cases the binder itself may suffice

for this purpose. This will occur when the binder is highly. . .
DETD . . . gel in the electroseparation medium to assist in providing localization of the complex bound to the binder and achieve appropriate **separation** of the free and bound species, i.e., the complex from the uncomplexed first and second sbp members. Subsequently, a determination. . .
DETD One specific embodiment of an **assay** in accordance with the present invention, by way of example and not limitation, is a membrane-receptor competitive binding **assay**.
DETD In this example, cell membrane receptors are attached to solid-phase **capture** media for facilitating the use of protein receptors in a microfluidic-based **assay**. Solid-phase attachment of the receptor is achieved in one of several ways, including, e.g., the use of activated paramagnetic beads. . .
DETD In the **assay** cellular membranes are harvested from tissues or cell cultures expressing the receptor of interest. The typical crude homogenate contains particles. . .
DETD The receptor-beads can be more uniformly dispensed based on their size, shape, and/or density. The process of transfer and/or **capture** is facilitated further by conferring a magnetic property to the beads.
DETD In this particular **assay** embodiment, activated paramagnetic beads of relatively uniform size are coupled to membrane receptors to deliver a predetermined quantity of target. . . antibodies or other binding moieties that selectively bind to the target molecule, including cells, genes, bacteria, or other biomolecules. This **assay** is particularly applicable for receptors belonging to the seven transmembrane family or similar proteins wherein the sequence of amino acids. . .
DETD A typical membrane-receptor competitive-binding **assay** in regard to the above is described next. The non-isotopic **assay** comprises two binding events. The primary receptor-ligand affinity reaction can be written generally as: ##EQU1## where the library test compound. . .
DETD A microfluidic **assay** device 100, configured generally as illustrated in FIG. 1, is employed in the **assay**. The components of microfluidic **assay** device 100 as illustrated in FIG. 1 are as follows: inlet reservoir 102 for buffer solution; inlet reservoir 104 for. . . solution; inlet reservoir 114 for solution used to cleave the fluorophore-tag from the fluorescent tracer conjugate; inlet reservoir 116 for **capture** compound; outlet reservoir 124 for waste solution from binding **assay** from the fluorescent tracer conjugate; outlet reservoir 126 for waste **capture** compound; outlet reservoir 128 for waste supernatant from binding; incubation, **separation** and detection chamber 125; secondary **capture** and detection chamber 135.
DETD Before outlining the **assay** protocol that is conducted on device 100 ("on-board"), reagent preparation conducted first ("on-line") includes the following:
DETD The bioanalytical **assay** proceeds on microfluidic device 100 as follows:
DETD . . . means of an applied magnetic field, to a "reaction chamber" 125 in fluid communication with reservoir 110. In this particular **assay** protocol, the beads are held in this reaction chamber for the duration of the procedure.
DETD . . . of compound and receptor, (R)-L.sup..cndot., to react with the streptavidin which binds biotin with high affinity. The amount of streptavidin **captured** is monitored directly for a fluorescent label, e.g., fluorescein, associated with the streptavidin.
DETD 4) In one form of this **assay**, the fluorescent label may be attached via a disulfide bond denoted by ":". This bond Is readily cleaved under reducing. . .
DETD 5) The fluorescent labeled species can then be **separated** from

other reactants by electrokinetic or hydrodynamic enhanced electroseparation techniques. To facilitate detection, the magnetic beads may be immobilized at. . . The fluorescent label may be detected in the fluorescent labeled species or the fluorescent label may be cleaved and detected **separately**.

DETD . . . for example, the invention may be applied to methods for DNA purification from whole blood and other samples, to mRNA **isolation**, to solid phase cDNA synthesis, to cell **isolation**, and so forth.

DETD Bioanalytical binding **assays** based on affinity reactions and multi-phase **separation** and/or localization methods are provided as examples of embodiments of the current invention. The tracer-binder **separation** step can include electrophoretic and/or biomagnetic processes. Experimental protocols are described that utilize synthetic particles to which an sbp member. . . site heterogeneity, more uniform flow distributions, format flexibility, and so forth. The use of hydrophilic low-binding surfaces, when combined with **capture** beads provides for a powerful method to minimize non-specific binding and to control the size of the resulting vesicles in the preparation of the crude receptor. In addition, the flexibility in **assay** design as afforded by microfluidic devices, when combined with solid-phase **capture** particles, is illustrated by way of a number of examples using various specific binding pairs and detection formats.

DETD FIG. 2 provides an illustration of a microfluidic device 200 configuration and method for carrying out a highly sensitive sandwich **immunoassay** that is readily amenable to high throughput clinical diagnostic applications. The sandwich **assay** reaction can be written generally as follows:

DETD . . . an incubation channel 225 for mixing and affinity binding followed by injecting a plug of the reaction mixture for electrophoretic **separation** and then detection of the sandwich complex, (Ab)-Ag-Ab*.

DETD Microfluidic device 200 may be utilized in performing sandwich immunodiagnostic **assays**. Device 200 comprises inlet reservoir 202 for antibody, Ab, specific to particular antigen wherein Ab is bound to plastic beads. . . 236 for detection product waste; injection cross 245; outlet reservoir 250 for waste; detection chamber/zone and detector 275; and secondary **capture** and detection chamber 236.

DETD An example of an immunodiagnostic competitive binding **assay** is based on the following affinity reaction:

DETD . . . of interest compete for the target antibody (Ab), which is attached to plastic beads of relatively uniform size. Because an **assay** protocol based upon this bioaffinity reaction is similar to the receptor-ligand competitive **assay**, it will not be further discussed here. The above protocol was used in the Example presented below.

DETD Receptor-ligand competitive binding **assays** employing microfluidic-based multi-phase monitoring schemes are also exemplary of the **assays** to which the present invention has application. Utilization of cell surface receptors for the discovery and characterization of pharmacologically active. . . of significant general interest in addition to being related to the methods according to this invention. Other embodiments include solid-phase **assays** for the purpose of characterizing particular **isolated** cell-membrane receptors. **Assay** protocols include those wherein a ligand labeled with a fluorophore, enzyme or biotin binds competitively with unlabeled ligand for the. . .

DETD In the above **assays** two distinct detection approaches arise when direct detection of both "bound" and "free" tracer is possible. Employing a microfluidic approach is particularly amenable to dual detection schemes. In one approach involving a **capture** **assay** for receptor-bound fluorophore-labeled ligand, detection

can be accomplished by solid-phase monitoring of "bound" tracer or downstream detection of cleaved fluorophore tag (Ab **capture** & measure enhanced signal).

DETD Instead of using commercially available (Sigma) particles or the methods provided above, alternative procedures for preparing solid-phase **capture** materials may be employed. More specifically, Concanavalin A-coated magnetic particles such as, e.g., JSR CIM-31 carboxy beads (0.8 micron in. . . A (ConA) is coated on the carboxy BSA particles. Once synthesized, the ConA-coated carboxy magnetic particles can be used to **capture** membrane-bound receptors while also minimizing the non-specific binding of receptor-selective fluorescent tracers. Depending on the particular **assay** protocol, this immobilization or **capture** step may be carried out prior to the binding **assay** or during the course of the **assay**.

DETD Examples of competitive receptor-ligand binding **assays** are described above in which pre-conjugated receptor-beads are employed. In another embodiment of the invention, solid-phase **capture** particles are introduced during the course of the bioanalytical **assay** to **separate** free and bound species. In this particular embodiment, receptor, tracer and test compound are allowed to incubate statically or in. . . a region of a microchannel in which beads are located. Having been synthesized for this purpose, the magnetic beads selectively **capture** the membrane-bound receptors. By applying a magnetic field, the beads are immobilized and the free fluorescent tracer in the supernatant is measured. In addition to this depletion **assay**, the bound fluorescent tracer can be measured in the solid phase or the **captured** fluorophore can be released using a detergent and then measured. More specifically, both formats have been demonstrated for a D2 GPCR **assay** employing a NAPS (from Molecular Probes)-tracer, which may be synthesized by known methods, D2 membrane receptors obtained from SF9 cells. . .

DETD Selective bead modifications have other beneficial attributes. More specifically, in capillary and chip-based heterogeneous enzyme **assay** such as, e.g., kinase **assays**, the enzyme substrate such as, e.g., cdc2 peptide (see, for example, Johnson, et al., J. Peptide Research (1997) 50:365-371), has been shown to interact with the surface of particular beads, yielding inhomogeneities that are detrimental to **assay** performance. To mask these inhomogeneities, beads have been coated with ConA. In a similar synthesis protocol as above, carboxy particles. . .

DETD Other examples of **assays** that may be carried out using the present invention are a nicotinic acetylcholine receptor **assay** using a fluorophore-labeled ligand and neuronal nicotinic acetylcholine receptor and ligand-gated ion channel. For the latter the **assay** procedure generally described above may be used to quantitatively characterize the interactions of cholinergic agents with the nicotinic acetylcholine receptor. . . cell transmembrane potential. Because neurostimulated excitation-contraction (EC) coupling plays an important role in synaptic transmission at the neuromuscular junction, nAChR **assays** are widely employed within the pharmaceutical industry for investigating lead compounds. More specifically, **assays** are used to characterize potential new drugs and assist in the understanding of their function. In addition, the nAChR can be used as a molecular recognition element in bioanalytical **assays** and sensors for detecting biological and chemical warfare agents.

DETD . . . addition to primary screening as described above, a microfluidic device similar to that of FIGS. 1 and 2 and multi-phase **assay** protocols utilizing the invention may be used for investigating further the **isolated** cell-membrane receptor. In one protocol a fixed concentration of ligand labeled with a fluorophore binds to the active binding sites. . .

DETD . . . of the "bound" response, B*=T*-F*, can be obtained by directly

measuring the fluoro-intensity of the bound ligand, B*, in the solid-**capture** phase and indirectly by measuring the fluoro-intensity of the background-corrected "total" tracer T* and the "free" tracer F* in the. . .

DETD . . . response as a function of a wide range of both tracer and receptor concentrations can be carried out to optimize **assay** performance.

DETD B. Dose-response analysis: Using a microfluidic-**assay** card device similar to those depicted in FIGS. 1 and 2, dose-response behavior for a given ligand-receptor system is obtained. . . the concentration of the test compound for a fixed amount of labeled ligand (tracer) and receptor. The fluorescent-based receptor binding **assay** described above may be used for investigating cholinergic drugs, mostly muscle relaxants. Labeled ligand, i.e., .alpha.-bungarotoxin fluorescein isothiocyanate conjugate (.alpha.-Bgt-FITC), binds competitively with unlabeled library compound using a sequential saturation procedure. After a **separation** step, the free label concentration is determined via LIF according to one of several **assay** protocols described below.

DETD Another **assay** of interest is a fluorescence-based receptor binding **assay** for investigating cholinergic ligands that interact with the .alpha.-binding site of the nAChR are presented. The **assay** system is based on sequential saturation of the cell-surface receptor by test compound L.sub.i and the fluorescein-labeled .alpha.-bungarotoxin (.alpha.-Bgt:FITC) tracer (see, e.g., Molecular Probes). The fluorescence-based receptor **assay** (FRA) may be used to obtain dose-response data for a number of cholinergic agents, including the agonist carbamylcholine and various. . .

DETD Another **assay** is a benzadiphine receptor competitive binding dual **assay** employing a biotinylated ligand and secondary SBP. A fluorometric receptor-based dual **assay** protocol may be used. The robust competitive-binding **assay** is particularly amenable to high-throughput pharmaceutical drug screening because "off-the-shelf" reagents are employed instead of having to synthesize special fluorophore-labeled ligand conjugates. The trade-off for universal reagents however, is a more complex dual **assay** protocol.

DETD In this embodiment of a dual **assay**, the first of two binding reactions is a primary receptor-ligand competitive binding reaction employing a biotinylated-ligand.

DETD . . . compound L.sub.i competes with the biotin-labeled drug L.sup..cndot. for the active binding sites of the bead-immobilized membrane receptor (R). After **separation** of the "free" from the "bound" L.sup..cndot. conjugate, the biotinylated drug in the supernatant from the first incubation step may. . . and/or depletion monitoring of the labeled ligand L.sup..cndot.. Protocols for each will be briefly outlined where a robust secondary binding **assay** is employed.

DETD The premise for the secondary **assay** is that the "free" biotinylated ligands L.sup..cndot. and the biotin beads compete for a limited number of biotin binding sites. . .

DETD . . . in the secondary binding reaction above, where * represents the fluorescent tag. After incubation, washing leaves only the fluorophore-labeled avidin **captured** onto the biotin solid phase.

DETD In addition to the "bound" detection-based **assay**, described above, "free" ligands in the supernatant can be electrokinetically moved downstream for detection by means of a secondary "on-board" **assay** protocol. In contrast to the more direct detection scheme and exemplified in the nAChR **assay** above, the concentration of free ligand in the suspension above the immobilized membrane receptor can be determined by a solid-phase **assay** utilizing a secondary

binding pair, e.g., avidin-biotin or streptavidin-biotin.

DETD . . . nervous system to produce hypnotic, muscle relaxing, tranquilizing and anticonvulsant activities and are widely prescribed in clinical practice. Highly sensitive **assay** methods to determine their concentration in tablets, blood, urine, etc. are required to evaluate their pharmacological effects and to investigate. . .

DETD . . . of many benzadiphine drugs or drug analogs, including clonazepam and lorazepam. In this embodiment of the invention, a benzadiphine receptor **assay** is described for the purpose of illustration only. Other receptor systems, including soluble receptors such as the interleukins, or cell surface receptors as part of whole cells, are also amenable to microfluidic-based **assays** similar to that presented here.

DETD A fluorometric receptor-based dual **assay** (FRDA) for the benzodiazepines clonazepam (O) and lorazepam (I) employing a biotinylated-drug probe and biotin-immobilized microfluidic card can yield dose-response. . .

DETD . . . represents membrane receptors attached to solid-phase particles, which can be immobilized by, for example, magnetic or electromagnetic means. The homogeneous **assay** does not require a **separation** step. Enzyme activity has been engineered such that its enzyme activity becomes inhibited upon binding with the receptor.

DETD As with the bead-based **assays** described above, the ERA method may be employed to investigate a wide variety of pharmacologically important receptors for their binding. . .

DETD In this example, a nicotinic acetylcholine receptor **assay** employing an enzyme-labeled ligand or fluorophore as the tracer is put forth as an example of a representative ERA system. An **assay** procedure for quantifying the binding of the hallucinogen phencyclidine (PCP), antipsychotic agents including tricyclic antidepressants (TCA's) and toxins all to. . . tracer. However, the drug screen has proven to be irreproducible due to its sensitivity to the experimental conditions of the **assay**, e.g., the heterogeneity associated with the distribution of the receptor. In contrast to the original work, the bead-based multi-**assay** format described herein provides for a bioanalytical screening method that is readily amenable to high throughput automation. In addition, masking. . .

DETD In principle, homogeneous **assays** are faster and easier to perform than heterogeneous **assays** because no bound/free **separation** step is necessary. However, due to matrix effects, it is typically difficult to measure a very low concentration in a complicated sample by homogeneous **assay**. In practice, a heterogeneous **assay** has a detection limit 100-fold lower than the most sensitive homogeneous **assay**. Nonetheless, in certain non-diagnostic applications, including pharmaceutical drug discovery, homogeneous **assays** offer sensitivity limits that are more than adequate for high-throughput primary screening. Thus, homogeneous **assays** hold much promise for their use in conjunction with microfluidic-based devices and methods.

DETD The present invention also has application to polynucleotide **assays** such as DNA **assays** and to other studies of polynucleotides such as hybridization studies and the like. For this purpose a highly-flexible, miniature, automated device capable of performing a broad range of DNA-based **assays** may be used. The multi-functional instrument provides for collecting DNA from a raw sample, amplifying the DNA using the polymerase chain reaction (PCR), and analyzing the DNA product by Taqman **assays**, fragment sizing, and ihybridization. Thus, on-chip fractionation of DNA from other cellular components may be conducted. In addition, the reusability. . . determining PCR product lengths can be achieved. A hybridization technique using surface-activated magnetic beads which can be reused for multiple **assays** may be conducted. All of these

DNA sample preparation and analysis features may be conducted in a single reusable laminated plastic microfluidic platform, using electro-osmotic pumping of fluids, external optical detection, and magnetic **capture** and release of magnetic beads.

DETD . . . by the control system. Sensors are read by the microprocessor and the information analyzed to determine the results of the **assay**. The system is readily amenable to later addition of telemetry devices to enable external control and transmission of results.

DETD . . . of spores/cells, DNA is extracted so that extraneous proteins and other biological matter does not interfere with the amplification and **separation** methods of the DNA. To accomplish this, micro-scaled bead-based methodologies upstream of the amplification and detection processes. For cases in. . .

DETD In the above approach, magnetic beads for the hybridization **assays** will be electroosmotically injected into the flow stream and trapped in incubation chambers using small permanent magnets mounted in close. . . flow path. Additional optical analysis can be made at that time or the beads can be introduced to some other **assay** such as off-chip flow cytometry. The expended beads can be released by moving the permanent magnet a short distance away from the bead chamber, and flushed to waste; new beads can be charged into the hybridization zone for additional **assays**. Several sample aliquots can be sent in parallel to different zones to test simultaneously for a number of different pathogens.. . .

DETD . . . an electroactive substance, and a capillary assay device. The reagents for the kits may be packaged in the same or **separate** containers, so that the concentration of the reagents provides for substantial optimization of the method and assay. The reagents may each be in **separate** containers or various reagents can be combined in one or more containers depending on the cross-reactivity and stability of the. . . is a sieving gel where the method includes the use of such a gel. The kit can further include other **separately** packaged material, ancillary reagents such as buffers, and so forth.

DETD Particle-based Sandwich **Assay**

DETD . . . I. The sizes of these particles range from 0.041 to 0.40 .mu.m. They all gave UV absorbance and no fluorescence. **Separation** conditions: capillary 27 cm long 50 .mu.m inside diameter (id) fused silica capillary; 7 cm effective **separation** distance. **Separation** buffer: 1 mM 2-[(2-amino-2-oxoethyl)amino]ethanesulfonic acid (ACES) buffer (pH 5.98). Injection: 3s at 8 kV. **Separation** voltage; 12 kV, 1.1 .mu.A, positive polarity. Detection UV absorbance at 254 nm.

DETD During CE all the particles were well **separated** from neutral marker (mesityl oxide) when the effective **separation** distance was 7 cm. The half-height peak width for 0.321 .mu.m particle was much wider than those of other particles.. . .

DETD The fluorescence of these particles was also tested using LIF detection. **Separation** conditions: capillary 47 cm long, 50 .mu.m id fused silica capillary; 7 cm effective **separation** distance. **Separation** buffer: I mM ACES buffer (pH 5.98). Injection: 3s at 8 kV. **Separation** voltage; 20 kV, 1.1 .mu.A, positive polarity. LIF detection. (A) buffer; (B) 0.041 .mu.m; C) 0.067 .mu.m (D) 0.11 .mu.m;. . . concentration. Therefore, polystyrene latex particles with the diameters of 0.041, 0.11 and 0.40 .mu.m were selected as potential candidates as **capture** antibody (unlabeled) carrier to form a sandwich complex with antigen and secondary antibody (fluorophore labeled).

DETD Anti-insulin monoclonal antibody (Cortex Biochem, San Leandro, Calif.) was employed as the **capture** antibody, which was physically adsorbed on the particle surface. Phosphate buffered saline (PBS) containing 100 mM phosphate, 150 mM NaCl. . .

DETD Mab-coated particles were analyzed by CE. Comparison of (A) Mab coated particles; (B) uncoated particles. **Separation** conditions: capillary 27 cm long, 50 .mu.m id using silica capillary; 20 cm active **separation** distance. **Separation** buffer: 5 mM phosphate buffer (pH 7.2). Injection: 1s at 2 kV for (A), 1s at 3 kV for (B). **Separation** voltage; 12 kV, 9 .mu.A, positive polarity. Detection UV absorbance at 214 nm. The migration time of coated beads was. . .

DETD 3. **Assay** between Mab Coated Particles and Insulin.

DETD The **assay** between Mab coated particles and FITC-insulin gave a binding curve, namely, the plot of peak height of complex between Mab-coated. . .

DETD . . . fixed amount of FITC-insulin solution. As the amount of Mab coated particles increased, amount of complex formation increased. A similar **assay** between free Mab and FITC-insulin was also performed.

DETD 4. **Separation** of Insulin Complexes.

DETD A series of experiments were carried out to improve the **separation** between the two complexes, i.e., insulin-Mab and insulin-Mab-coated particles. The **separation** of these two complexes provides a direct indication of the separability of the sandwich complex Mab-particles-insulin-FITC-Mab') from free labeled antibody under the same conditions. The effects of the buffer's ionic strength, **separation** distance and applied voltage were Investigated. In this investigation the higher the ionic strength, the worse the **separation** and the longer the **separation** distance, the better the **separation**. The experimental results demonstrated that these two complexes were separable.

L10 ANSWER 3 OF 55 USPATFULL
ACCESSION NUMBER: 2001:14210 USPATFULL
TITLE: Nucleic acid mediated electron transfer
INVENTOR(S): Meade, Thomas J., Altadena, CA, United States
Kayyem, Jon Faiz, Pasadena, CA, United States
Fraser, Scott E., La Canada-Flintridge, CA, United States
PATENT ASSIGNEE(S): California Insitute of Technology, Pasadena, CA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6180352	20010130
APPLICATION INFO.:	US 1999-459191	19991210 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-306749, filed on 7 May 1999 Continuation of Ser. No. US 1997-873598, filed on 12 Jun 1997, now patented, Pat. No. US 5952172 Continuation of Ser. No. US 1996-660534, filed on 7 Jun 1996, now patented, Pat. No. US 5770369 Continuation of Ser. No. US 1995-475051, filed on 7 Jun 1995, now patented, Pat. No. US 5824473 Continuation of Ser. No. US 1993-166036, filed on 10 Dec 1993, now patented, Pat. No. US 5591578
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Zitomer, Stephanie W.
LEGAL REPRESENTATIVE: Flehr Hohbach Test Albritton & Herbert LLP; Trecartin, Esq., Richard F.; Silva, Esq., Robin M.
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 36 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT: 2493
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides for the selective covalent modification of nucleic acids with redox active moieties such as **transition metal** complexes. Electron donor and electron acceptor moieties are covalently bound to the ribose-phosphate backbone of a nucleic acid at predetermined positions. The resulting complexes represent a series of new derivatives that are bimolecular templates capable of transferring electrons over very large distances at extremely fast rates. These complexes possess unique structural features which enable the use of an entirely new class of bioconductors and photoactive probes.

AB The present invention provides for the selective covalent modification of nucleic acids with redox active moieties such as **transition metal** complexes. Electron donor and electron acceptor moieties are covalently bound to the ribose-phosphate backbone of a nucleic acid at predetermined. . .

SUMM The detection of specific nucleic acid sequences is an important tool for diagnostic medicine and molecular biology research. Gene probe **assays** currently play roles in identifying infectious organisms such as bacteria and viruses, in probing the expression of normal genes and. . .

SUMM Ideally, a gene probe **assay** should be sensitive, specific and easily automatable (for a review, see Nickerson, Current Opinion in Biotechnology 4:48-51 (1993)). The requirement. . .

SUMM Specificity, in contrast, remains a problem in many currently available gene probe **assays**. The extent of molecular complementarity between probe and target defines the specificity of the interaction. Variations in the concentrations of. . .

SUMM . . . in the reaction conditions will alter the hybridization. New experimental techniques for mismatch detection with standard probes include DNA ligation **assays** where single point mismatches prevent ligation and probe digestion **assays** in which mismatches create sites for probe cleavage.

SUMM Finally, the automation of gene probe **assays** remains an area in which current technologies are lacking. Such **assays** generally rely on the hybridization of a labeled probe to a target sequence followed by the **separation** of the unhybridized free probe. This **separation** is generally achieved by gel electrophoresis or solid phase **capture** and washing of the target DNA, and is generally quite difficult to automate easily.

SUMM The time consuming nature of these **separation** steps has led to two distinct avenues of development. One involves the development of high-speed, high-throughput automatable electrophoretic and other **separation** techniques. The other involves the development of non-**separation** homogeneous gene probe **assays**.

SUMM For example, Gen-Probe Inc., (San Diego, Calif.) has developed a homogeneous protection **assay** in which hybridized probes are protected from base hydrolysis, and thus are capable of subsequent chemiluminescence. (Okwumabua et al. Res.. . . 143:183 (1992)). Unfortunately, the reliance of this approach on a chemiluminescent substrate known for high background photon emission suggests this **assay** will not have high specificity. EPO application number 86116652.8 describes an attempt to use non-radiative energy transfer from a donor. . .

SUMM As outlined above, molecular biology relies quite heavily on modified or labeled oligonucleotides for traditional gene probe **assays** (Oligonucleotide Synthesis: A Practical Approach. Gait et al., Ed., IRL Press: Oxford, UK, 1984; Oligonucleotides and Analogues: A Practical Approach.. . .

SUMM . . . properties of the ruthenium label (Telser et al., J. Am. Chem. Soc. 111:7221 (1989)). Other experiments have successfully added two **separate** spectroscopic labels to a single double-stranded DNA molecule (Telser et al., J. Am. Chem. Soc. 111:7226 (1989)).

SUMM . . . in Inorganic Chemistry: Bioinorganic Chemistry, Vol. 38, Ed. Stephen J. Lippard (1990). In addition, the selective modification of metalloenzymes with **transition metals** has been accomplished and techniques to monitor electron transfer in these systems developed. For example, electron transfer proteins such as. . .

SUMM The present invention provides for the modification of nucleic acids at specific sites with redox active moieties such as **transition metal** complexes. An electron donor and/or electron acceptor moiety are covalently bound at predetermined positions. The resulting complexes represent a series. . .

DRWD . . . moieties EDM and EAM on two adjacent single stranded nucleic acids. These orientations also apply when the two probes are **separated** by an intervening sequence.

DRWD FIG. 5 is a schematic showing **transition metals** bound to the ribose-phosphate backbone in a variety of positions. M is a **transition metal**. M.sub.1 is bound via an amine on the 2' carbon of the ribose; an electron must travel through 4 .sigma.. . .

DRWD . . . nucleoside to control pore glass (CPG) and the formation of a single stranded nucleic acid with elongation and attachment of **transition metal** complexes as the exemplified electron transfer species. The experimental conditions are outlined in Example 9.

FIG. 6A depicts the formation. . . a 3' and 5'2'-amino modified

nucleoside. FIG. 6C depicts the addition of the electron transfer species, exemplified by two ruthenium **transition metal** complexes, im(bpy).sub.2 Ru and Ru(II)(NH.sub.3).sub.4 py.

DRWD FIG. 7 depicts the addition of electron transfer moieties, exemplified by a **transition metal** complex, to the C-terminus of PNA. FIG. 9 attaches 4-aminomethylpyridine to the carboxy terminus, to form a ligand which may. . .

DETD . . . to utilize a number of compounds in the present invention. Preferred electron transfer moieties include, but are not limited to, **transition metal** complexes, organic electron transfer moieties, and electrodes.

DETD In a preferred embodiment, the electron transfer moieties are **transition metal** complexes. **Transition metals** are those whose atoms have an incomplete d shell of electrons. Suitable **transition metals** for use in the invention include, but are not limited to, cadmium (Cd), magnesium (Mg), copper (Cu), cobalt (Co), palladium. . . (Cr), manganese (Mn), nickel (Ni), Molybdenum (Mo), technetium (Tc), tungsten (W), and iridium (Ir). That is, the first series of **transition metal**, the platinum metals (Ru, Rh, Pd, Os, Ir and Pt), along with Re, W, Mo and Tc, are preferred. Particularly. . .

DETD The **transition metals** are complexed with a variety of ligands to form suitable **transition metal** complexes, as is well known in the art. Suitable ligands include, but are not limited to, --NH.sub.2 ; pyridine; pyrazine;. . .

DETD In addition to **transition metal** complexes, other organic electron donors and acceptors may be covalently attached to the nucleic acid for use in the invention.. . .

DETD . . . materials. This strong attachment is included in the definition of "covalently attached" for the purposes of this embodiment. The epoxide **cross-linking polymer** is then reacted with, for example, an exposed amine, such as the amine of an amino-modified nucleic acid described above,. . .

DETD . . . can be substituted for the redox enzyme or mediator with the result of electron transfer processes being observed from a **transition metal**-modified DNA moiety through a coupled redox conducting polymer to an electrode.

DETD . . . of organic salts, cobaltocenes, the hexa- and octacyanides of molybdenum, tungsten and iron. In addition, macrocycles and chelating ligands of **transition metals** such as cobalt, ruthenium and nickel are used, including Co(ethylenediamine).sub.3 and Ru(ethylenediamine).sub.3 and the trisbypyridyl and hexamine complexes of **transition metals** such as Co, Ru, Fe, and Os (see Alyanasundaram, supra).

DETD Thus, all combinations of electron donors and acceptors may be made: two **transition metal** complexes; two organic electron transfer species; one **transition metal**, one organic moiety; one **transition metal** and an electrode; and one organic moiety and an electrode. The choice of the electron transfer species will depend in. . .

DETD . . . target domain may be directly adjacent to the second target domain, or the first and second target domains may be **separated** by an intervening target domain. The terms "first" and "second" are not meant to confer an orientation of the sequences. . .

DETD The present invention is directed, in part, to the site-selective modification of nucleic acids with redox active moieties such as **transition metal** complexes for the preparation of a new series of biomaterials capable of long distance electron transfer through a nucleic acid. . .

DETD . . . the target sequence as compared to when no amplification occurs. A particular advantage of the present invention is that the

separation of the single stranded primers from the amplified double stranded DNA is not necessary, as outlined above for probe sequences. . .

DETD The modified nucleosides are then used to site-specifically add a **transition metal** electron transfer moiety, either to the 3' or 5' termini of the nucleic acid, or to any internal nucleoside.

Either. . .

DETD . . . the 2' or 3' position of the ribose and thus used to attach the

electron transfer moiety such as a **transition metal** complex. This may effectively reduce the number of .sigma. bonds an electron must travel through to reach the "pi-way" since. . .

DETD . . . ribose, resulting in an internal attachment. For example, phosphoramide rather than phosphodiester linkages can be used as the site for **transition metal** modification. These **transition metals** serve as the donors and acceptors for electron transfer reactions. While structural deviations from native

phosphodiester linkages do occur and. . .

DETD The cleavage from the CPG may occur either prior to **transition metal** modification or afterwards.

DETD . . . significantly perturbed in order to allow hybridization, good electron transfer rates, and the detection of mismatches. Thus, for example, the **transition metal** moieties, when attached to the nucleic acids of the invention, do not intercalate, i.e.

insert and stack between the basepairs of the double stranded nucleic acid. Intercalation of the **transition metals** with the accompanying ligands disturbs the basepairing, and thus hinders the transfer of electrons and the identification of mismatches. Similarly, with the exception of terminal bases, as is outlined below, attaching the **transition metal** complexes at the nucleoside bases (Telser et al., supra) also disturbs the basepairing and impedes the identification of mismatches.

DETD As described above, the electron transfer moiety, preferably a **transition metal** complex, may be attached to any of the five bases (adenine, thymine, uracil, cytosine, guanine and other non-naturally occurring bases. . .

DETD . . . the other linkages useful in the present invention, are readily

modified with a variety of electron transfer moieties, and particularly **transition metal** complexes with techniques readily known in the art (see for example Millet et al, in Metals in Biological Systems, Sigel. . . Washington D.C.; and Meade et al., J. Am. Chem. Soc. 111:4353 (1989)). Generally, these techniques involve contacting a partially chelated **transition metal** complex with the amine group of the modified nucleoside.

DETD . . . electron transfer moieties proceeds as follows. The amino group

at the N-terminus of the PNA will bind a partially chelated **transition metal** or organic electron transfer moiety similar to the amino-modified ribose. Addition to the carboxy terminus can proceed in a variety. . .

DETD . . . to an unmodified complementary sequence. This blocks the sites on the heterocyclic bases that are susceptible to attack by the **transition metal** electron transfer species.

DETD After successful addition of the desired metal complex, the modified duplex nucleic acid is **separated** into single strands using techniques well known in the art.

DETD . . . for distances in excess of 100 nucleosides, a preferred embodiment has the electron donor moiety and the electron acceptor moiety **separated** by at least 3 and no more than 100 nucleosides. More preferably the moieties are **separated** by 8 to 64 nucleosides, with 15 being the most preferred distance.

DETD . . . protected amino-modified nucleoside is then removed, and the

hybrid is contacted with the second electron transfer species, and the strands **separated**, resulting in a single strand being labeled with both a donor and acceptor. The single strand containing the proper electron. . .

DETD . . . this manner, which may result in extremely long range transfer of electrons. This may be accomplished, for example, by incorporating **transition metal** complexes that possess a range in oxidation potentials due to ligand substitutions made at the metal center.

DETD . . . complexes therefore include acridine orange, N,N'-dimethyl-2,7-diazapyrenium dichloride (DAP.sup.2+), methylviologen, ethidium bromide, quinones such as N,N'-dimethylanthra(2,1,9-def:6,5,10-d'e'f')diisoquinoline dichloride (ADIQ.sup.2+); porphyrins ([meso-tetrakis(N-methyl-x-pyridinium)porphyrin tetrachloride]. **Transition metal** donors and acceptors include complexes of ruthenium, rhenium and osmium (most preferred) where at least one of the ligands is. . .

DETD . . . electron donor moiety on the DNA via an inter-molecular process. Such mediators include water soluble and stable complexes of the **transition metals**, including molybdenum and tungsten halides, trisbipyridyl complexes of rhenium, osmium and ruthenium. In addition, other examples include bipyridyl and pyridyl complexes such as Re(bpy)(CO).sub.3 X where X is a halide and Re(py).sub.4 O.sub.2. Other examples include **transition metal** dimers such as [Re.sub.2 Cl.sub.8].sup.2- and [Pt.sub.2 (P.sub.2 O.sub.5 H.sub.2).sub.4].sup.4-. Ruthenium trisbypyridine (Ru.sup.2+ (bpy).sub.3) is most preferred.

DETD . . . cobaltocenes, the hexa- and octacyanides of molybdenum, tungsten and iron. In addition, other examples include macrocycles and chelating ligands of **transition metals** such as cobalt, ruthenium and nickel, including Co(ethylenediamine).sub.3 and Ru(ethylenediamine).sub.3 and the trisbypyridyl and hexamine complexes of **transition metals** such as Co, Ru, Fe, and Os. See K. Alyanasundaram, Coord. Chem. Rev. V.46, p. 159, 1982. Finally, organic molecules. . .

DETD . . . on the choice of electron acceptors attached to the nucleic acid. High driving forces are achieved using bisbipyridyl complexes of **transition metals**, for example, ruthenium and rhenium bisbipyridyl complexes such as (Ru(bpy).sub.2 im-) as electron acceptors.

DETD In a preferred embodiment, the electron transfer is detected fluorometrically. Numerous **transition metal** complexes, including those of ruthenium, have distinct fluorescence properties. Therefore, the change in redox state of the electron donors and. . .

DETD Many **transition metal** complexes display fluorescence with large Stokes shifts. Suitable examples include bis- and trisphenanthroline complexes and bis- and trisbipyridyl complexes of **transition metals** such as ruthenium (see Juris, A., Balzani, V., et. al. Coord. Chem. Rev., V. 84, p. 85-277, 1988). Preferred examples. . .

DETD . . . currently used to monitor blood glucose, for example. This method of detection involves applying a potential (as compared to a **separate** reference electrode) between the nucleic acid-conjugated electrode and an auxiliary (counter) electrode in the sample containing target genes of interest.. . .

DETD In accordance with a further aspect of the invention, the preferred formulations for donors and acceptors will possess a **transition metal** covalently attached to a series of ligands and further covalently attached to an amine group as part of the ribose. . .

DETD . . . to the complementary unmodified strand using standard techniques. All manipulations of the annealed duplex, prior to the addition of the **transition metal** complex were handled at 4.degree. C. In order to insure that the DNA remained

annealed during modification, the reactions were. . .

DETD . . . equipped with a stirring bar and septum was slurried Ru(III) tetraaminepyridine chloride (10 .mu.m), in the same buffer. In a **separate** flask, Zn/Hg amalgam was prepared and dried under reduced pressure and the ruthenium(III) solution transferred (via cannulation) to the Zn/Hg. . .

DETD The amplified template sequences with **transition metal** complexes on both 5' termini are purified by agarose gel electrophoresis and used directly in electron transfer applications.

DETD . . . is followed by quantiative ninhydrin analysis (Sarin, Anal. Biochem., 117:147 (1981)). The resulting PNA may be modified with an appropriate **transition metal** complex as outlined in example 1.

DETD . . . The dimer units are substituted for standard oligonucleotides at chosen intervals during the preparation of DNA using established automated techniques. **Transition metal** modification of the modified linkages takes place as described in Example 1.

DETD . . . base at the predicted retention time and characteristic UV-vis spectra was confirmed. An identical procedure was carried out on the **transition metal** modified duplex DNA and assignments of constituent nucleosides demonstrated single-site modification at the predicted site.

DETD . . . temperature controlled UV-vis (Hewlett-Packard), using techniques well known in the art. These results confirm that hybridization of the amino-modified and **transition metal** modified DNA had taken place. In addition, the results indicate that the modified DNA form a stable duplex comparable to. . .

DETD . . . Rev. 92:369-379 (1992). The donor is Ru(bpy).sub.2 (NHuridine)im, E.sup.0.about.1 V, and the acceptor is Ru(NH.sub.3).sub.4 py(NHuridine)im, E.sup.0.about.330 mV. The purified **transition metal** modified oligonucleotides (U.sub.NHRu(bpy)2im GCATCGA and U.sub.NHRu(NH3)4(py)im CGATGCA were annealed by heating an equal molar mixture of the oligonucleotides (30 .mu.molar:. . .

DETD . . . reaction is terminated by heat inactivation of the enzyme at 75.degree. C. for 10 minutes. The doubly labeled oligonucleotide is **separated** from the singly labeled oligonucleotides and the complementary unlabeled oligonucleotide by HPLC in the presence of urea as in the. . .

DETD . . . the polymerase chain reaction (Saiki et al., Science 239:487-491 (1988)). This region of HIV-I is highly conserved among different clinical **isolates**.

DETD . . . in FIG. 6A. The modified oligonucleotide were assembled by standard solid phase automated DNA synthesis techniques and the bis-3',5',-2'-amino-2'-deoxyuridine oligonucleotide **isolated** and characterized by mass spectrometry and HPL(, digestion analysis. In addition, the aminoribose oligomers and their complements were reacted with. . .

CLM What is claimed is:

10. A method according to claim 1 or 3 wherein said electron donor moiety is a **transition metal** complex.

11. A method according to claim 10 wherein said **transition metal** complex comprises a metal selected from the group consisting of ruthenium, rhenium, osmium, platinum, copper and iron.

L10 ANSWER 5 OF 55 USPATFULL

ACCESSION NUMBER: 2000:168200 USPATFULL

TITLE: Phosphatase activated crosslinking conjugating and reducing agents; methods of using such agents; and reagents comprising phosphatase activated crosslinking and conjugating

INVENTOR(S): Bieniarz, Christopher, Highland Park, IL, United States

Young, Douglas F., Grayslake, IL, United States
Cornwell, Michael J., Morton Grove, IL, United States
Skrzypczynski, Zbigniew, Vernon Hills, IL, United States

PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6160153	20001212
APPLICATION INFO.:	US 2000-498388	20000203 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1996-657695, filed on 29 May 1996, now patented, Pat. No. US 6057429 which is a division of Ser. No. US 1994-349167, filed on 2 Dec 1994, now patented, Pat. No. US 5736624

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Ceperley, Mary E.
LEGAL REPRESENTATIVE: Anderson, Regina M.
NUMBER OF CLAIMS: 2
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 44 Drawing Figure(s); 18 Drawing Page(s)
LINE COUNT: 1787
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides crosslinking, conjugating and reducing agents which are functional with at least one phosphorothioate monoester group (--SPO.sub.3.sup.--2). Crosslinking and conjugation methods as well as solid phase reagents and conjugates which are useful in immunoassays are also provided.

Crosslinking and conjugating agents of the invention generally comprise a compound corresponding to the formula (I), shown below, wherein n at least 1 and Q is a straight or branched monomer, polymer or oligomer having an average molecular weight between about 200 and about 1,000,000. Additionally, when n is 1, Q comprises at least 1 additional reactive functionality.

Q--(S--PO.sub.3.sup.-2).sub.n (I)

The reducing agents that are provided conform to a compound of the formula (Y), shown below, wherein (A) and (Z) can be independently selected from C.sub.1 -C.sub.5 alkyl and CONH(CH.sub.2).sub.p wherein p is an integer between 1 and 5. ##STR1##

SUMM . . . bound on the support and the solid support, having the first binding member, the analyte and conjugate bound thereon is **separated** from any unbound conjugate, typically with one or more wash steps. In the case of an enzyme immunoassay, an indicator. . .

DETD . . . The solid phase can be chosen for its intrinsic ability to attract and immobilize a binding member to form a **capture** reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize a binding member to form a **capture** reagent. The additional receptor can include a charged substance that is oppositely charged with respect to a binding member or. . . receptor molecule enables the indirect binding of a binding member to a solid phase material before the performance of the **assay** or during the performance of the **assay**. The solid phase thus can be a latex, plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface or. . .

DETD . . . acid and carboxylic acids, mixed cellulose esters, and cellulose ethers; natural polymers containing nitrogen, such as proteins and derivatives, including **cross-linked** or modified gelatins; natural hydrocarbon **polymers**, such as latex and rubber; synthetic polymers which may be prepared with suitably porous

structures, such as vinyl polymers, including. . .

DETD . . . activity relevant for its intended use. Thus, for example, if a stabilized compound is a binding member used in an **immunoassay**, it will have the capacity to bind its complementary binding member to form a binding pair; if a stabilized compound. . .

DETD . . . samples can be used such as water, food products and the like for the performance of environmental or food production **assays**. In addition, a solid material suspected of containing the analyte can be used as the test sample. In some instances,. . .

DETD . . . as methacrylates, quinone groups, and epoxide groups; thiopyridyl groups; as well as other protected disulfides such as, for example, cysteamine; **transition metal** complexes or **transition metals** in various oxidation states or in colloidal forms which are known to form stable coordinate bonds with thiols such as,. . .

DETD . . . day 0 and various time points along the course of the study the activity of the dilutions was evaluated. To **separate** 1.0 ml volumes of 7 mM PNPP in buffer F was added 20 .mu.l of the 10 .mu.g/ml ALP dilutions.. . .

DETD Three 5 .mu.l aliquots of 8.2 mM aqueous solution of cysteamine-S-phosphate were placed into three **separate** vials. The three samples were diluted to 1 ml with 0.1 M sodium acetate buffer, pH 4.0. The final pH. . .

DETD . . . added to the resulting white residue and the mixture was stirred for 10 minutes. A white precipitate formed which was **separated** from the supernatant liquid and dried under reduced pressure to yield the powder product N-hydroxysuccinimidyl cysteamidophosphorothioate 4,5-dithioheptyl 1-carboxylate.

DETD . . . to the resulting white residue and the resulting mixture was stirred for 10 minutes. A white precipitate formed and was **separated** from the supernatant liquid and dried under reduced pressure to yield the product N-hydroxysuccinimidyl cysteamidophosphorothioate 3-oxybutyl 1-carboxylate as a white. . .

L10 ANSWER 13 OF 55 USPATFULL
ACCESSION NUMBER: 2000:54212 USPATFULL
TITLE: Phosphatase activated crosslinking, conjugating and reducing agents; methods of using such agents; and reagents comprising phosphatase activated crosslinking and conjugating agents
INVENTOR(S): Bieniarz, Christopher, Highland Park, IL, United States
Young, Douglas F., Grayslake, IL, United States
Cornwell, Michael J., Morton Grove, IL, United States
Skrzypczynski, Zbigniew, Vernon Hills, IL, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6057429	20000502
APPLICATION INFO.:	US 1996-657695	19960529 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1994-349167, filed on 2 Dec 1994, now patented, Pat. No. US 5736624
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Ceperley, Mary E.
LEGAL REPRESENTATIVE: Anderson, Regina M.
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 1,6
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 18 Drawing Page(s)
LINE COUNT: 1776
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides crosslinking, conjugating and reducing agents which are functional with at least one phosphorothioate monoester group (--SPO.sub.3.sup.-2). Crosslinking and conjugation methods as well as solid phase reagents and conjugates which are useful in immunoassays are also provided.

Crosslinking and conjugating agents of the invention generally comprise a compound corresponding to the formula (I), shown below, wherein n at least 1 and Q is a straight or branched monomer, polymer or oligomer having an average molecular weight between about 200 and about 1,000,000. Additionally, when n is 1, Q comprises at least 1 additional reactive functionality.

Q--(S--PO.sub.3.sup.-2).sub.n (I)

The reducing agents that are provided conform to a compound of the formula (Y), shown below, wherein (A) and (Z) can be independently selected from C.sub.1 -C.sub.5 alkyl and CONH(CH.sub.2).sub.p wherein p is an integer between 1 and 5. ##STR1##

SUMM . . . bound on the support and the solid support, having the first binding member, the analyte and conjugate bound thereon is **separated** from any unbound conjugate, typically with one or more wash steps. In the case of an enzyme immunoassay, an indicator. . .

DETD . . . The solid phase can be chosen for its intrinsic ability to attract and immobilize a binding member to form a **capture** reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize a binding member to form a **capture** reagent. The additional receptor can

include a charged substance that is oppositely charged with respect to a
binding member or. . . receptor molecule enables the indirect binding
of a binding member to a solid phase material before the performance of the **assay** or during the performance of the **assay**.
The solid phase thus can be a latex, plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface or. . .

DETD . . . acid and carboxylic acids, mixed cellulose esters, and cellulose ethers; natural polymers containing nitrogen, such as proteins
and derivatives, including **cross-linked** or modified gelatins; natural hydrocarbon **polymers**, such as latex and rubber, synthetic polymers which may be prepared with suitably porous structures, such as vinyl polymers, including. . .

DETD . . . activity relevant for its intended use. Thus, for example, if a
stabilized compound is a binding member used in an **immunoassay**, it will have the capacity to bind its complementary binding member to form a binding pair; if a stabilized compound. . .

DETD . . . samples can be used such as water, food products and the like for the performance of environmental or food production **assays**. In addition, a solid material suspected of containing the analyte can be used as the test sample. In some instances,. . .

DETD . . . as methacrylates, quinone groups, and epoxide groups; thiopyridyl groups; as well as other protected disulfides such as, for example, cystamine; **transition metal** complexes or **transition metals** in various oxidation states or in colloidal forms which are known to form stable coordinate bonds with thiols such as,. . .

DETD . . . day 0 and various time points along the course of the study the
activity of the dilutions was evaluated. To **separate** 1.0 ml volumes of 7 mM PNPP in buffer F was added 20 .mu.l of the 10 .mu.g/ml ALP dilutions.. . .

DETD Three 5 .mu.l aliquots of 8.2 mM aqueous solution of cysteamine-S-phosphate were placed into three **separate** vials.
The three samples were diluted to 1 ml with 0.1 M sodium acetate buffer,
pH 4.0. The final pH. . .

DETD . . . added to the resulting white residue and the mixture was stirred for 10 minutes. A white precipitate formed which was **separated** from the supernatant liquid and dried under reduced pressure to yield the powder product N-hydroxysuccinimidyl cysteamidophosphorothioate 4,5-dithioheptyl 1-carboxylate.

DETD . . . to the resulting white residue and the resulting mixture was stirred for 10 minutes. A white precipitate formed and was **separated** from the supernatant liquid and dried under reduced pressure to yield the product N-hydroxysuccinimidyl cysteamidophosphorothioate 3-oxybutyl 1-carboxylate as a white. . .

L10 ANSWER 14 OF 55 USPATFULL

ACCESSION NUMBER:	2000:12671 USPATFULL		
TITLE:	Systems for surface-enhanced affinity capture for desorption and detection of analytes		
INVENTOR(S):	Hutchens, T. William, Davis, CA, United States Yip, Tai-Tung, Davis, CA, United States		
PATENT ASSIGNEE(S):	Baylor College of Medicine, Houston, TX, United States (U.S. corporation)		
	NUMBER	DATE	
PATENT INFORMATION:	US 6020208	20000201	
	WO 9428418	19941208	
APPLICATION INFO.:	US 1995-556951	19951127	(8)
	WO 1994-US6064	19940527	

19951127 PCT 371 date
19951127 PCT 102(e) date

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Alexander, Lyle A.
LEGAL REPRESENTATIVE: Fulbright & Jaworski L.L.P.
NUMBER OF CLAIMS: 54
EXEMPLARY CLAIM: 38
NUMBER OF DRAWINGS: 44 Drawing Figure(s); 42 Drawing Page(s)
LINE COUNT: 2559
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention is directed to systems containing probes for presenting an analyte to an energy source for desorption in methods of analytic detection, such as mass spectrometry. The probes have an immobilized affinity reagent which binds the analyte on their presenting surface.

SUMM . . . costly, particularly because SDS polyacrylamide gel electrophoresis is an adequate substitute in some instances where MALDI would be applied (e.g., **separation** of crude biological fluids). In addition, MALDI has had little exposure in biological and clinical journals.

DETD . . . strong or "permanent" bonds resulting from true electron sharing), coordinate covalent bonds (e.g., between electron donor groups in proteins and **transition metal** ions such as copper or iron), and hydrophobic interactions (such as between two noncharged groups).

DETD . . . atoms in biomolecules (e.g, N, S, O) "donate" or share electrons with electron poor groups (e.g., Cu ions and other **transition metal** ions).

DETD . . . of probe elements (i.e., sample presenting means) with Surfaces Enhanced for Laser Desorption/Ionization (SELDI), within which there are three (3) **separate** subcategories. Surfaces Enhanced for Neat Desorption (SEND) where the probe element surfaces (i.e., sample presenting means) are designed to contain. . .

DETD . . . may be deliberately cut or fragmented by chemical and/or enzymatic means so that many of the resulting fragments are now **separate** and distinct analytes, each one still attached (tethered) to the probe surface by one or more photolabile bonds, to be. . .

DETD . . . said analyte molecules. In a further embodiment, said analyte molecules are biomolecules and said affinity reagent is adapted to selectively **isolate** said biomolecules from an undifferentiated biological sample. In a preferred embodiment, said matrix materials are in the weakly acidic to. . .

DETD . . . preferred embodiment presents analyte molecules are biomolecules and said affinity capture device or photolabile attachment molecule is adapted to selectively **isolate** said biomolecules from an undifferentiated biological sample.

DETD . . . is released in a light dependent manner; or, where said analyte molecules are biomolecules, said PAM is adapted to selectively **isolate** said biomolecules from an undifferentiated biological sample. In another preferred embodiment, said matrix materials are in the weakly acidic to. . .

DETD These surfaces can be derivatized (at varying densities) to bind by chemical bonds (covalent or noncovalent) affinity adsorption reagents (affinity **capture** devices), energy absorbing molecules (bound "matrix"molecules) or photolabile attachment molecules. The geometry of the sample presenting surface is varied (i.e.,. . . thickness, etc.) to suit the need (e.g., insertion into a living organism through spaces of predetermined sizes) of the experiment (**assay**).

DETD Surface Enhanced Affinity **Capture**, SEAC

DETD This example describes the use of existing and new solid phase affinity reagents designed for the (1) **capture** (adsorption) of one or

more analytes, (2) the preparation of these **captured** analytes (e.g., washing with water or other buffered or nonbuffered solutions to remove contaminants such as salts, and multiple cycles. . . polar organic solvent, detergent-dissolving solvent, dilute acid, dilute base or urea), and (3) most importantly, the direct transfer of these **captured** and prepared analytes to the probe surface for subsequent analyte desorption (for detection, quantification and/or mass analysis). Affinity **capture** devices are immobilized on a variety of materials, including electrically insulating materials (porous and nonporous), flexible or nonrigid materials, optically. . . sample surface, for selective adsorption/presentation of sample for mass analysis are (1) stainless steel (or other metal) with a synthetic **polymer** coating (e.g., **cross-linked** dextran or agarose, nylon, polyethylene, polystyrene) suitable for covalent attachment of specific biomolecules or other nonbiological affinity reagents, (2) glass. . .

DETD I. Surface Immobilized Metal Ion as the Affnity **Capture** Device

DETD . . . IDA-Cu(II) at pH 7.0 (20 mM sodium phosphate, 0.5M sodium chloride) at 23.degree. C. for 10 min. The gel is **separated** from the remaining peptide solution by centrifugation and is then washed with 200 .mu.l sodium phosphate, sodium chloride buffer, pH. . .

DETD . . . IDA-Cu(II) at pH 7.0 (20 mM sodium phosphate, 0.5M sodium chloride) at 23.degree. C. for 10 min. The gel is **separated** from the solution by centrifugation and then washed with 500 .mu.l of buffer two times and 500 .mu.l of water. . .

DETD II. Surface immobilized antibody as the affinity **capture** device

DETD . . . affinity-adsorbed on surface immobilized antibody (if the analyte signal is unambiguously identified in a mixture of primary antibody-analyte complex, any **capture** device, e.g., surface immobilized secondary antibody, Protein A, Protein G, Streptavidin, of the primary antibodies is used in this method. . . via specific molecular recognition events where one of the analytes is detected through its association with the primary target of **capture;** and c) the use of magnetic surface as efficient **capture** device.

DETD . . . device on a flat surface (a two-dimensional configuration) of a flexible probe element. This SEAC device may be used to **isolate** target analyte materials from undifferentiated biological samples such as blood, tears, urine, saliva, gastrointestinal fluids, spinal fluid, amniotic fluid, bone. . .

DETD . . . to the analyte. One way of doing this is by the combination of enzyme catalysis and the streptavidin-biotin system. After **capturing** minute quantities of lactoferrin on a nylon probe element as described in Example 3.II.2. biotinylated anti-lactoferrin antibody or biotinylated single-stranded. . . of amplification comes from the enzyme catalysis where the enzyme can achieve a turnover number of 10.sup.2 to 10.sup.3 min.sup.-1. **Assay** of alkaline phosphatase enzyme activity can easily be accomplished by using a low molecular weight phosphorylated substrate such as ATP,. . .

DETD . . . improvement of detection at the present moment is achieved by the amplification based on the polymerase chain reaction principle. After **capturing** minute quantities of lactoferrin on a nylon probe element as described in Example 3.II.2. biotinylated anti-lactoferrin antibody or biotinylated single-stranded. . .

DETD . . . spectrometry in the presence of sinapinic acid. Then the semipure preparation of human FSH (Chemicon) is digested with trypsin and **separate** aliquots (7 ul) are reacted with the immobilized antibodies (10 ul of 1:1 gel suspension) in phosphate-buffered saline at

4.degree.. . .
DETD III. Surface Immobilized Nucleic Acid as the Affinity **Capture** Device
DETD . . . acid are suspended in water and the pH is adjusted to 6.6 with dilute sodium hydroxide. Tentacle DEAE Fractogel (EM **Separations**, Gibbstown, N.J.) is washed with 20 mM HEPES, pH 6.0 and suction dried.
The energy absorbing molecules solution is mixed. . .
CLM What is claimed:
. . . a material selected from the group consisting of an electrically insulating material, a flexible material, an optically transparent material, a **cross-linked polymer** and a biopolymer.

52. The method of claim 50 wherein the material is a **cross-linked polymer** or a biopolymer.

L10 ANSWER 16 OF 55 USPATFULL

ACCESSION NUMBER:	1999:159840 USPATFULL
TITLE:	Magnetically assisted binding assays utilizing a magnetically responsive reagent
INVENTOR(S):	Rohr, Thomas E., Gurnee, IL, United States Elstrom, Tuan A., Lake Bluff, IL, United States Howard, Lawrence V., Libertyville, IL, United States Shain, Eric B., Glencoe, IL, United States
PATENT ASSIGNEE(S):	Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5998224	19991207	
APPLICATION INFO.:	US 1997-857440	19970516	(8)
DOCUMENT TYPE:	Utility		
PRIMARY EXAMINER:	Stucker, Jeffrey		
LEGAL REPRESENTATIVE:	Weinstein, David L.		
NUMBER OF CLAIMS:	44		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	27 Drawing Figure(s); 25 Drawing Page(s)		
LINE COUNT:	2878		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Assay methods utilizing the response of a magnetically responsive reagent to the influence of a magnetic field to qualitatively or quantitatively measure binding between specific binding pair members. According to the invention, the presence of an analyte mediates whether or not the magnetically responsive reagent binds to a mobile solid phase reagent. The extent of binding will modulate the response of the magnetically responsive reagent or that of the mobile solid phase reagent, or both, to the influence of a magnetic field. Hence, by measuring the response to the magnetic field of the magnetically responsive reagent, or that of the mobile solid phase reagent, the presence or amount of analyte contained in a test sample can accurately be determined. The invention utilizes various devices to carry out the assay methods described.
SUMM . . . More particularly, the invention relates to the use of magnetically responsive materials to change the properties of components in binding **assays**.
SUMM Diagnostic **assays** have become an indispensable means for detecting analytes in test samples by using the mutual reaction between the analyte and. . . tags or labels attached to antibodies, which in turn bind to the analyte of interest, are employed in such diagnostic **assays**, wherein the detection of the resultant labeled antibody-analyte complex, or detection of the labeled antibody that does

not bind to. . .

SUMM Two commonly used diagnostic **assay** techniques employing specific binding members are the **radioimmunoassay** (RIA) and the enzyme **immunoassay** (EIA), both of which employ a labeled specific binding member. The RIA uses a radioactive isotope as the detectable tag. . .

SUMM . . . and conjugate to binding members, and may be unstable during storage at room temperature or even under refrigerated conditions. Enzyme **immunoassays** are also unsatisfactory in that the methods typically require complex incubations, multiple liquid additions, and multiple wash steps.

SUMM More recently, **assay** techniques using metallic sol particles as visual labels have been developed. In these techniques, a metal (e.g., gold, silver, platinum),. . . particles. Generally, the specific binding member to be labeled is adsorbed onto the metallic sol particles, and the particles are **captured** or aggregated in the presence of analyte. Although the metallic sol particles have the advantage of producing a signal that. . . difficult to measure quantitatively. The metallic sol particles also have a limited color intensity, and consequently, limited sensitivity in some **assays** . In addition, the surfaces of inorganic metallic sol particles, such as gold, may not readily accept the covalent attachment of specific binding members. Thus, during use in a binding **assay**, care must be taken so that the adsorbed specific binding members are not removed from the inorganic particles through the. . .

SUMM Self-performing **immunoassay** devices have proven to be of great benefit in the field of diagnostics. A self-performing **immunoassay** device is a kit containing immunoreagents to which a biological sample can first be added by the patient or laboratory technician, then the diagnostic **assay** performed without the need for complex laboratory instruments. Commercially available self-performing **immunoassay** devices, such as the strip **assay** device having the trademark "TESTPACK PLUS", distributed by Abbott Laboratories, enable **immunoassays** to be performed quickly and reliably.

SUMM Typically, self-performing **immunoassay** devices involve chromatographic test strips. For example, U.S. Pat. No. 4,960,691 discloses a test strip for analysis of an analyte. . .

SUMM A problem with **assay** devices of this type is the inherent variability in the material from which the porous matrix is formed. This variability. . . example, in porosity) directly affects the flow of fluid through the matrix and may adversely affect the precision of the **assay** device. Furthermore, the matrix will often non-specifically bind the particles or reagents at sites at the intended reaction zone or. . . passivating procedures after the immobilized reagent has been applied. Consequently, there is a desire to develop a rapid, simple, self-performing **assay** device that does not require a fluid to flow through a porous matrix.

SUMM Another problem with self-performing **immunoassay** devices is the necessity of immobilizing a specific binding reagent on the test strip so that reagents involved in the **assay** can be **captured** at the reaction zone. The process of immobilizing the specific binding reagents on the test strip can be difficult to. . . of the reaction zone to change after shipping or storage. Because the immobilized specific binding reagent is specific for the **assay** of a particular analyte, test strips must be dedicated to a particular **assay**. An additional problem with self-performing **immunoassay** devices is lot-to-lot variation resulting from manufacturing processes, especially variation of the activity of the biological reagents, such as the binding molecules. For example, lot-to-lot variations in the binding capacity of the binding reagent at

the **capture** zone of a test strip can affect **assay** results. Although adjustments in the activities or concentrations of the other reagents can compensate, making such adjustments involves introducing undue. . . lot of test strips to particular lots of reagents. The ability to use a completely stable, uniform test strip in **assays** for several different analytes would greatly simplify the production and control of strip-based self-performing **assays**. Alternatively, the ability to readily adapt a test strip during manufacturing to meet the requirements of a set of reagents. . .

SUMM In several applications it is desirable to use a self-performing **assay** which gives a positive result above a certain analyte concentration and a negative result below that concentration, with a very. . .

SUMM Superparamagnetic microparticles are also used extensively in the performance of **immunoassays**. Superparamagnetic microparticles are magnetically responsive in that an applied magnetic field will cause a force to act upon them in. . . the application of a magnetic field and the material not bound to the conjugate is removed (commonly known as bound/free **separation**), as described in U.S. Pat. Nos. 4,745,077; 4,070,246; and 3,985,649 . Additional wash steps, reagent additions, and bound/free **separations** are usually required before a measurable signal is produced. Analytical methods of this type typically use light emission (chemiluminescence or. . . analyte of interest. Typically, the magnetic responsiveness of the superparamagnetic particles is used only as an aid in the bound/free **separation** steps, with the remainder of the **assay** procedure involving conventional reagents and protocols. Consequently, conventional analyses using superparamagnetic particles are limited to either complex automated instrumentation (for example, the ACS 180 from Ciba Corning Diagnostics) or an extended series of manual **assay** steps.

SUMM . . . individual particles. The strength and gradient of the applied magnetic field can also be selected to favor the movement or **capture** of particular types or forms of magnetically responsive reagents.

SUMM U.S. Pat. No. 5,108,933 discloses a method whereby colloidal, magnetically responsive particles can be used for the **separation** of any one of a variety of target substances from a test medium suspected of containing the substance of interest. . . test medium, forming a magnetic agglomerate comprising the colloidal particles and any target substance present in the test medium, and **separating** the resulting magnetic agglomerates from the medium. This method of analysis, however, uses only a single type of particle, thereby. . .

SUMM . . . that bring about formation of rosettes consisting of magnetic particles, non-magnetic fluorescent particles, and the desired cells. The rosettes are **separated** from the non-magnetic components of the test sample by application of a magnetic field, whereupon the number of cells can. . .

SUMM . . . interest in the sample is determined. In this process, the presence of analyte is not detected by directly observing the **separated** magnetic/nonmagnetic particle complexes near the location of the magnet.

SUMM U.S. Pat. Nos. 5,445,970 and 5,445,971 describe the use of a magnetically-attractable material as a detectable label in binding **assays**. The magnetic label is subjected to a magnetic field and the label, in turn, displays a resultant force or movement. . . phase, or on the free magnetically-attractable material, then reflects the quantity of analyte present in the test mixture. Although self-performing **assay** formats are possible using this approach, specific **capture** on some form of non-mobile solid phase is required.

SUMM For some applications, an **assay** format using only mobile solid

phases such as microparticles would have distinct advantages, as would formats that do not require. . . stable suspensions, called ferrofluids, are only weakly attracted to the source of a magnetic field and therefore cannot be readily **captured** magnetically. Ferrofluids also are usually not compatible with aqueous solutions. It would be advantageous to develop self-performing **immunoassay** formats that do not require a chromatographic material. It would also be advantageous to develop a medium for a self-performing **immunoassay** that could be used for a multiplicity of **immunoassays** and easily adapted to reagent variations resulting from manufacturing processes.

SUMM . . . a magnetic force is exerted upon said complex, the influence of said magnetic force being manifested by the movement or **capture** of said complex at a different rate from that of said magnetically responsive reagent alone or from that of said. . .

SUMM . . . a magnetic force is exerted upon said complexes, the influence of said magnetic force being manifested by the movement or **capture** of said second complex at a different rate from that of said magnetically responsive reagent alone or from that of. . .

SUMM . . . a specified threshold, the amount of complex containing both specific binding members formed will be below the threshold of the **assay**.

SUMM . . . a specified threshold, the amount of complex containing both specific binding members formed will be below the threshold of the **assay**.

SUMM . . . or the variation in force exerted upon the source of the magnetic field by the reagents during or following magnetic **separation** of the complexes;

SUMM (2) a visual device for measuring the extent of complex formation by magnetic **separation** of unbound reagents from reagents in complexes;

SUMM (3) a visual device, or an optical device, for measuring (a) the extent of complex formation by magnetic **capture** of magnetically responsive reagent bound to a mobile solid phase reagent and the **separation** of unbound magnetically responsive reagent or the mobile solid phase reagent or (b) both reagents by movement in a capillary. . .

SUMM . . . by measuring perturbation of a magnetic field caused by changes in the distribution of the complexes during or following magnetic **separation** of the complexes;

SUMM . . . extent of complex formation by measuring the variation in the optical density of the reaction mixture during or following magnetic **separation** of the complexes;

SUMM . . . by measuring the change in reflectivity of an optically reflective surface due to the force exerted upon it by magnetically **captured** complexes during or following magnetic **separation** of the complexes.

SUMM In an embodiment of a self-performing **immunoassay** device that can be used to replace a conventional strip device for performing **immunoassays**, specific binding members similar to those fixed to the porous matrix of a conventional self-performing **immunoassay** device are fixed to particles of magnetically responsive material, e. g., superparamagnetic particles, and the resulting magnetically responsive reagent is. . . an optical device and indicates the presence or amount of analyte in the sample. It should also be noted that **assays** utilizing the principles of this invention can also be conveniently carried out in conventional reaction vessels, e. g., cuvettes, wells,. . .

SUMM A particular advantage of this invention is the ease with which an **immunoassay** may be performed by means of a hand-held, self-contained device. The magnetic field of ordinary magnetic recording

tape or credit card magnetic strips is sufficient to cause the **separation** of complexes containing magnetically responsive reagent from diamagnetic mobile solid phase reagent. The presence of these complexes may easily and. . . diamagnetic solid phase material within the complexes. Another particular advantage of this invention is the ability to create a magnetic **capture** zone that will be matched to the magnetically responsive reagents and mobile solid phase reagents employed. The magnetic field and its gradient can be defined so as to provide optimal attraction of the magnetically responsive reagent.

The magnetic **capture** site(s) can be used to provide semi-quantitative readings by visual means in self-performing **assays**. The magnetic **capture** site(s) can be controlled to provide means to compensate for lot-to-lot variations in **assay** reagents. As stated previously, the reagents used in conventional binding **assays** are usually complex biologic mixtures and tend to vary from one lot to another because of manufacturing processes. For sandwich **assay** formats, where particles of the magnetically responsive reagent may be very small relative to the particles of the mobile solid. . . magnetically responsive reagent by controlling the strength and gradient of the magnetic field. It is also possible to control the **capture** of the particles of the mobile solid phase reagent that may be large relative to the particles of the magnetically responsive reagent. In order to most efficiently **capture** the complexes comprising particles of mobile solid phase reagent and particles of magnetically responsive reagent without **capturing** the unbound particles of magnetically responsive reagent, it is possible to provide a field gradient that changes with distance comparable. . .

DRWD FIG. 14A is a schematic view of one type of self-performing **immunoassay** device in operation for the magnetically assisted detection of complexes containing a magnetically responsive reagent. The figure depicts the **immunoassay** before the complex is **captured**.

DRWD FIG. 14B is a schematic view of the self-performing **immunoassay** device of FIG. 14A in operation for the magnetically assisted detection of complexes containing a magnetically responsive reagent. The figure

DETD . . . analyte complexes, or assay reagents become bound and from which unreacted assay reagents, test sample, or test solutions can be **separated**. The solid phase generally has a specific binding member attached to its surface to form a "solid phase reagent", that.

.

DETD . . . ancillary binding member can be incorporated into the assay device or it can be added to the device as a **separate** reagent solution.

DETD . . . magnetic field on the individual complexes suspended in the fluid is relatively high, and consequently, much more readily detectable. The **separation** of individual particles from complexes and movement of complexes in a magnetic field forms the basis for the method and. . .

DETD . . . can comprise a core formed of a single particle of a magnetically responsive material having a coating of a water-insoluble, **cross-linked polymeric** material that has reactive groups at the surface thereof. A layered particle can comprise a core of a non-magnetic material. . .

DETD . . . form of iron and a water-soluble polymer having available coordination sites (free electron pair for a coordinate bond with a **transition metal** atom); (e) an organic, inorganic, or synthetic polymeric matrix containing a magnetically responsive material; (f) a continuous phase of a. . .

DETD . . . has not undergone a specific binding reaction to form a complex (i. e., the unbound magnetically responsive reagent) can be **separated** from the magnetically responsive reagent that has undergone a specific binding reaction to form a complex (i. e., the bound. . . responsive reagent) due to their different behavior in an applied magnetic field. It will be understood, of course, that the **separation** of the bound magnetically responsive reagent and the unbound magnetically responsive reagent may involve the complete removal of the unbound. . .

DETD The **separation** of the bound magnetically responsive reagent and the unbound magnetically responsive reagent may also involve the segregation of the unbound. . .

DETD Generally, devices according to the present invention comprise components for performing magnetically assisted binding **assays** as taught herein. Accordingly, such devices preferably comprise (i) a reaction vessel; (ii) a magnetic field generator for the application.

.

DETD The reaction vessel can be any device capable of containing the **assay** reagents disclosed herein and where the unbound magnetically responsive reagent and the bound magnetically responsive reagent can be produced in. . .

DETD **Separating** the bound magnetically responsive reagent from the unbound magnetically responsive reagent can be accomplished by any means suitable for partitioning. . .

DETD . . . magnets and electromagnets. It will also be understood, of course, that the magnetic field generator may also be used to **separate** the unbound or free magnetically responsive reagent from the bound or complexed magnetically responsive reagent.

DETD . . . magnetic field, the change in stress will be detectable. It will be understood, of course, that depending upon the particular **assay**, it may be preferred to detect, directly or indirectly, the response of the unbound magnetically responsive reagent, the

response of. . .

DETD The present invention solves the problems of conventional heterogeneous and **agglutination assays** by allowing the magnetically responsive reagent to associate with like reagents or with other magnetically non-responsive particles, then applying a. . . field, and measuring the consequences of the magnetic force exerted upon the magnetically responsive reagent to provide qualitative or quantitative **assay** results. Small levels of force can be readily determined using detectors which include, but are not limited to, electronic balances;. . . used to sense force changes in an atomic force microscope; and the like. These detectors enable performance of very sensitive **assays** and obviate the need for amplification of the label, as is required in many conventional **assays**.

DETD Conventional heterogeneous binding **assays** require vigorous washing of the solid phase to **separate** bound labeled reagent and unbound labeled reagent and to suppress the nonspecific binding of materials to the solid phase. Such wash steps complicate the **assay** protocol and restrict the **assay** to the use of specific binding pair members having high affinity, i.e., a binding strength that will withstand such physical manipulation. In conventional particle **agglutination assays**, binding members of low affinity can be used because several binding sites on each member can cooperate to give high avidities, and the absence of wash steps allows weak associations to be maintained while simplifying the **assay** format. Signal amplification results because the interaction of a few binding sites can cause the aggregation of complexes several orders. . . and mass than the original binding members, and thereby provide a macroscopic change, which can be interpreted visually. However, particle **agglutination assays** are often difficult to interpret, do not yield quantitative results, and are not readily amenable to automation.

DETD . . . gradients or both onto magnetically responsive materials. A field intensity or gradient or both that is optimal for a particular **assay** and particular binding reagents can be chosen, thereby allowing for correction of lot-to-lot variations in other reagents, or in the selective binding of certain subsets of reagents or complexes so as to obtain more precise **assay** results. It is to be understood that the aforementioned advantages permit the **assays** to be readily adapted to control by computer.

DETD While various devices and **assay** protocols are contemplated by the present invention, the following protocols represent examples, and are not limited to, a sandwich **assay** format and an indirect/competitive **assay** format using magnetically assisted detection of a magnetically responsive reagent. In this regard, the following protocols, and protocols contemplated by. . .

DETD . . . the magnetically responsive reagent bound to the solid phase, the influence of this force being manifested by the movement or **capture** of the complexes containing the magnetically responsive reagent and the mobile solid phase reagent at a different rate from that. . .

DETD The following embodiments exemplify how the method of the present invention can be used to perform **immunoassays**.

DETD As a further embodiment of the present invention, FIGS. 14A and 14B illustrate a self-performing **immunoassay** device for performing analytical tests. The device 600 comprises a capillary channel 602 having one or more magnetic sites 604.. . .

DETD . . . the analyte, and the magnetically responsive reagent having different ratios of mobile solid phase reagent and magnetically responsive reagent are **captured** at different site(s) 604 as an indication of concentration of analyte in the test sample.

DETD . . . optimize qualitative, e. g., positive/negative, results or more quantitative, e. g., semi-quantitative, results. For example, a series

of identical magnetic **capture** sites could be encoded along the bottom of the capillary channel illustrated in FIGS. 14A and 14B. Each site could. . . As the reaction mixture progresses downstream along the channel, the complexes would first encounter the most upstream of the magnetic **capture** sites of the series and be accumulated there. In a reaction mixture containing few complexes, only the most upstream of the magnetic **capture** sites would display accumulation of complexes. If, however there were sufficient complexes to saturate the most upstream of the magnetic **capture** sites, additional complexes would flow past that site to be accumulated at the next most upstream of the magnetic **capture** sites, and so on. The number of magnetic **capture** sites displaying accumulation of complexes would then serve as a measure of the extent of complex formation in the reaction. . .

DETD Alternatively, in certain **assay** formats, the concentration of the analyte in the reaction mixture can be manifested by the number of particles of magnetically. . . each particle of mobile solid phase reagent rather than by the extent of complex formation. In these cases, multiple magnetic **capture** sites, which differ in magnetic field strength or gradient or both, can be encoded on the bottom of the capillary. . . phase reagent. For example, as the reaction mixture progressed downstream along the channel, the complexes could first encounter the magnetic **capture** site having the weakest field intensity. This site would only **capture** those complexes having a high ratio of magnetically responsive reagent to mobile solid phase reagent. Subsequent magnetic **capture** sites would progressively increase in field intensity, thereby being capable of **capturing** complexes having lower and lower ratios of magnetic responsive reagent to mobile solid phase reagent. In this case, which magnetic **capture** site(s) of the series display accumulation of complexes will be an indication of how extensive the accumulation of the magnetically. . .

DETD . . . responsive reagent can be masked, which is a property especially useful in formats where the magnetically responsive reagent is always **captured** at the magnetic **capture** site.

DETD In another embodiment of the present invention, FIGS. 15A and 15B illustrate a self-performing **immunoassay** device for performing analytical tests. The device 700 comprises an element 702 having a flat surface 704 containing one or. . . In some applications, it may be advantageous to choose the intensity or gradient of the magnetic field at the magnetic **capture** site(s), or the size and composition of the magnetically responsive reagent, so that the magnetically responsive reagent accumulates whether it. . .

DETD . . . the analyte, and the magnetically responsive reagent having different ratios of mobile solid phase reagent and magnetically responsive reagent are **captured** at different sites 706 as an indication of concentration of analyte in the test sample. It is to be understood. . .

DETD In another embodiment of the present invention, FIGS. 16A and 16B illustrate a self-performing **immunoassay** device for performing analytical tests. The device 800 comprises a reaction vessel 802 supported above a magnet 804. The reaction. . .

DETD . . . and eluted with the same buffer. The ferrofluid eluted as a brown band in the excluded volume of the column. **Separation** on a column of "SEPHACRYL S-500" gel filtration media (exclusion limit 2.times.10.sup.7 daltons) resulted in the ferrofluid being partially included (see FIG. 20A), whereas **separation** on a column of "SEPHACRYL S-1000" gel filtration media (exclusion limit >10.sup.8 daltons) resulted in most of the particles being. . .

DETD The linear rate of the weight changes over the three minute periods of observation indicated that **capture** of the complexes was not completed in this time. Over longer periods of observation, the more rapid weight change of. . .

DETD . . . were capable of binding sufficient numbers of ferrofluid particles to the polypyrrole latex particles to allow the complexes to be **captured** magnetically, the use of more concentrated

ferrofluid suspensions resulted in a higher loading of ferrofluid on the
polypyrrole latex.
DETD Inhibition **Assay** for Free Biotinylated BSA
DETD Diluted solutions of free biotinylated BSA were made with an **assay** diluent obtained from Abbott Laboratories to give concentrations of 0 .mu.g/ml, 2 .mu.g/ml, 10 .mu.g/ml, and 40 .mu.g/ml. For each. . . was in place was noted. As shown in FIG. 24, increasing
concentrations of free biotinylated bovine serum albumin inhibits the **capture** of the ferrofluid by the polypyrrole latex, with the result that fewer complexes are formed and less ferrofluid accumulates at. . .
DETD Fabrication of Device Having Capillary Channels for Self Performing **Immunoassays**
DETD **Capture** of Polypyrrole Latex at a Magnetic Site in a Capillary Channel as a Result of Complex Formation with Ferrofluid
DETD . . . order to determine the optimal concentration of ferrofluid required to confer magnetic responsiveness upon polypyrrole latex for use in an **assay** format employing a device having capillary channels, the biotinylated BSA coated ferrofluid from the column containing "SEPHAROSE S-300" gel filtration. . . 25A and 25B). All channels corresponding to test samples containing the diluted solutions of ferrofluid showed a black band of **captured** polypyrrole latex at the position of the magnetic strip, the intensity of the band increasing as the concentration of the. . .
DETD Qualitative Inhibition **Assay** for Free Biotinylated BSA Performed Using a Device Having Capillary Channels
DETD Four **separate** solutions containing 0 .mu.g, 5 .mu.g, 20 .mu.g, and 80 .mu.g of the biotinylated BSA described in Example 4 in. . . described in Example 5. The presence of free biotinylated BSA at a concentration of 5 .mu.g/ml or more inhibited the **capture** of the polypyrrole latex at the magnetic site of the channels.
DETD Quantitative Inhibition **Assay** for Free Biotin Performed Using a Device Having Capillary Channels
DETD Diluted solutions of the biotinylated BSA described in Example 4 were made in **assay** diluent to concentrations of 0 .mu.g/ml, 5 .mu.g/ml, 10 .mu.g/ml, 20 .mu.g/ml, and 80 .mu.g/ml. A 20 .mu.l aliquot of. . . described in Example 5. The presence of free biotinylated BSA
at a concentration of 5 .mu.g/ml or more inhibited the **capture** of polypyrrole latex at the magnetic site of the channels.
DETD Quantitative results could be obtained for this type of **assay** by measuring the change in reflectance of the magnetic **capture** site due to the presence of **captured** polypyrrole latex. The **assay** was repeated as above, except that biotinylated BSA concentrations of 0.125 .mu.g/ml, 1.0 .mu.g/ml, 5.0 .mu.g/ml, and 80 .mu.g/ml were. . . the capillary channels, the channels were scanned using a scanning reflectance reader fabricated at Abbott Laboratories. Reflectance at the magnetic **capture** site was compared to the reflectance of sites on either side of the magnetic **capture** site and the net reflectance calculated as the difference between the reflectance at the magnetic **capture** site and the average of the two neighboring sites. The difference between the reflectance at the
magnetic **capture** site and the neighboring, non-magnetic sites decreased as the concentration of free biotinylated BSA increased, because the free biotinylated BSA inhibited binding between the polypyrrole latex and the ferrofluid (see FIG. 26). The **assay** response shows the narrow range desirable for a positive/negative **assay** format.
DETD A Self-Performing Spot **Immunoassay** for Human Chorionic Gonadotropin Coating Ferrofluid with Antibody
DETD . . . pH 7.0 with HCl, to a concentration of 1% solids. All further steps with the ferrofluid up to the final **assay** were performed in this buffer. Soluble material and particles without attached iron

were removed from the ferrofluid preparation by applying. . .

DETD A self-performing **assay** for soluble fibrin in human plasma was developed. The **assay** was based on the rate of optical density change of a suspension of polypyrrole latex coated with a specific binding. . . standard and the stock polypyrrole solution. The stock polypyrrole suspension was diluted twelve fold with diluent for use in the **assay**.

DETD The **assay** reagent mixture was prepared by mixing 1 ml of the diluted antibody coated polypyrrole suspension with 100 .mu.l of the.

.

.

DETD **Assay** standards were prepared from human soluble fibrin (obtained from American Biogenetic Sciences) diluted to different concentrations with the diluent solution.

DETD A Cary 3 spectrophotometer was used to determine the optical density of the **assay** mixture. A 5 mm inside diameter.times.30 mm fluorometer cuvette was obtained from Wilmad Glass Inc. A hex-head cap screw was. . . rested upon the top of the magnet. The reference cuvette holder was similarly blocked. The instrument was zeroed before the **assay** was begun.

DETD The **assay** was performed by first mixing 50 .mu.l of the **assay** reagent mixture with 50 .mu.l of the test mixture and then transferring the resulting mixture to the cuvette. The cuvette. . . density data by the spectrophotometer begun immediately. The rate of clearance of the polypyrrole latex from the solution by magnetic **capture** was reflected in the rate of change of the optical density of the suspension. A plot of the rate of optical density change in the **assay** mixture as a function of soluble fibrin concentration in the test sample shows a linear relationship (see FIG. 27).

L10 ANSWER 22 OF 55 USPATFULL
ACCESSION NUMBER: 1999:15462 USPATFULL
TITLE: Magnetic-polymer particles
INVENTOR(S): Owen, Charles S., Swarthmore, PA, United States
Silvia, John C., Lindenwold, NJ, United States
D'Angelo, Louis, Berlin, NJ, United States
Liberti, Paul A., Churchville, PA, United States
PATENT ASSIGNEE(S): Nycomed Imaging AS, Oslo, Norway (non-U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5866099	19990202
APPLICATION INFO.:	US 1997-846575	19970430 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-231323, filed on 22 Apr 1994 which is a continuation of Ser. No. US 1992-971513, filed on 3 Nov 1992, now abandoned which is a continuation of Ser. No. US 1988-245351, filed on 16 Sep 1988, now abandoned which is a continuation of Ser. No. US 1986-906521, filed on 16 Sep 1986, now patented, Pat. No. US 4795698 which is a continuation-in-part of Ser. No. US 1985-784863, filed on 4 Oct 1985, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Hollinden, Gary E.
LEGAL REPRESENTATIVE: Lyon & Lyon LLP
NUMBER OF CLAIMS: 1
EXEMPLARY CLAIM: 1
LINE COUNT: 674

AB A magnetic-polymer particle, useful in immunoassay techniques and various other biological/medical applications is produced by coprecipitation of **transition metals** in the presence of a polymer having available coordination sites. These particles are capable of forming stable aqueous suspensions and may be easily resuspended after agglomeration.

AB A magnetic-polymer particle, useful in immunoassay techniques and various other biological/medical applications is produced by coprecipitation of **transition metals** in the presence of a polymer having available coordination sites. These particles are capable of forming stable aqueous suspensions and. . .

SUMM . . . active magnetic particles may find use in a variety of preparative and diagnostic techniques. Among these is high gradient magnetic **separation** (HGMS) which uses a magnetic field to **separate** magnetic particles from suspension. In instances where these particles are attached to biological materials of interest (e.g. cells, drugs), the material of interest may thereby be **separated** from other materials not bound to magnetic particles.

SUMM . . . An atom in a molecular structure which has a "free" electron pair capable of forming a coordinate bond with a **transition metal** atom.

SUMM . . . the preparation of suspendable and resuspendable magnetic-polymer particles and the particles produced thereby. Such particles exhibit useful properties, particularly in **immunoassays** wherein the particles are prepared with a particular biofunctional ligand and are subsequently **separated** by high gradient magnetic **separation** techniques.

SUMM . . . various biofunctional groups may be incorporated into the particles in order to yield an effective biofunctional reagent for use in **immunoassay**, cell **capture**, enzyme immobilization

reactors, NMR imaging, and other diagnostic and analytical techniques.

SUMM . . . than iron in the coprecipitation reaction. In particular, Fe (III) may be replaced by any of a wide range of **transition metal** ions. In some cases, iron may be completely supplanted by appropriately selected **transition metal** ions. In many cases, the sue of metals other than ion produces colored particles ranging from white to dark brown.

SUMM Subsequent to precipitation and resuspension of the magnetic-polymer particles, they may be treated with a bifunctional reagent in order to **cross-link** reactive sites present on the **polymer**. This **cross-linking** may be effective as either an intro-particulate cross-linking in which reactive sites are bound on the same particle, or may be a reaction of an extra-particulate ligand which is then **cross-linked** to the **polymer** on a given particle. In the second case, a bifunctional reagent having a relatively short distance between its two functional. . .

SUMM In selecting the **transition metals** to be employed in the coprecipitation reaction, several criteria appear to be important. First, the final compound must have one. . .

DETD In addition to the list above, Fe(II) may be used in combination with selected **transition metal** ions whose electromotive potential is insufficient to oxidize the Fe(II) to Fe(III). Of the above listed metals, only V(III) is. . .

DETD . . . minutes 3.1 mg dithiothreitol (DTT) was added to convert the SPDP to its free sulfhydryl form. The reacted Ab was **separated** (desalted) on a small gel filtration column.

DETD Demonstration of Magnetic **Immunoassay** Using Particles Coupled to an Antigen

DETD . . . containing enzyme substrate. After 15 minutes incubation, the buffer was eluted and the amount of reaction product generated by enzyme **captured** on the filter bed was determined by measuring the optical density.

DETD The above procedure formed the basis of a competitive **immunoassay** for human IgM. When small amounts of free IgM were added to the incubation mixture, the uptake of enzyme by. . . the incubation mixture was graphed to produce a calibration curve which allowed the process to be used as a competitive **immunoassay** to measure unknown amounts of IgM. The sensitivity was approximately 0.15 mg/ml (the concentration of IgM which resulted in 50% reduction in the specific **capture** of enzyme activity on the filter).

DETD . . . goat-anti-rabbit-Ig activity by hemagglutination. A series of microtiter wells was set up containing sheep red blood cells (SRBC) and a sub-**agglutinating** concentration of rabbit antibody against SRBC. In each series, particles were added in concentrations which decreased two-fold in each successive well in the series. After several hours, the maximum well in which **agglutination** could be seen was read for each series. More active particles **agglutinated** at lower concentrations (larger well numbers). A table of results for the 12 preparations tested is given below.

DETD v) The particles, when coupled to specific biofunctional ligands, may be used in magnetic **immunoassays** for biological materials of interest.

DETD . . . novel magnetic-polymer particles and methods for making them. These particles are useful in a variety of biological/medical fields including cell **capture**, use as a contrast reagent for NMR imaging, immobilized enzyme reactors, **immunoassay**, and other analytical and diagnostic techniques.

CLM What is claimed is:

1. A method of **separating** a target substance from a non-target substance within a fluid mixture, comprising the steps of: combining the

fluid mixture with. . . produced by a process comprising the steps of: (a) combining a first aqueous solution of at least two species of **transition metal** ions capable of reacting with each other to form a magnetic precipitate and a polymer having available coordination sites in proportions adapted to produce a resuspendable product; (b) reacting said **transition metal** ions in the presence of said polymer to form a magnetic precipitate comprising magnetic-polymer particles; and (c) recovering said magnetic-polymer.

. magnetic field; attracting the magnetic particles toward a collection surface; and collecting the magnetic component upon the collection surface, thereby **separating** the target substance from the non-target substance.

L10 ANSWER 23 OF 55 USPATFULL

ACCESSION NUMBER:	1999:4337 USPATFULL
TITLE:	Bio-oligomer libraries and a method of use thereof
INVENTOR(S):	Lam, Kit Sang, Tucson, AZ, United States Salmon, Sydney E., Tucson, AZ, United States
PATENT ASSIGNEE(S):	The Arizona Board of Regents, Tucson, AZ, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5858670	19990112
APPLICATION INFO.:	US 1996-735623	19961023 (8)

RELATED APPLN. INFO.:	Continuation of Ser. No. US 1991-717454, filed on 19 Jun 1991, now patented, Pat. No. US 5650489 which is a continuation-in-part of Ser. No. US 1990-546845, filed on 2 Jul 1990, now abandoned
DOCUMENT TYPE:	Utility
PRIMARY EXAMINER:	Sisson, Bradley L.
LEGAL REPRESENTATIVE:	Pennie & Edmonds LLP
NUMBER OF CLAIMS:	13
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	12 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT:	2915

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The instant invention provides a library of bio-oligomers of defined size and known composition, in which the library contains all of the possible sequences of the bio-oligomers, and a method of synthesis thereof. The bio-oligomers of the library may be peptides, nucleic acids, or a combination of the foregoing. The instant invention also provides methods to identify bio-oligomers from a library that demonstrate desired characteristics such as binding, bioactivity and catalytic activity. Thus the instant invention provides a unique and powerful method to identify a useful bio-oligomer sequences from a library more quickly than current state-of-the-art technology allows. Effector molecules for use in treatment or diagnosis of disease are also provided.

SUMM . . . The sheer number and variety-of such peptide sequences has made this an unattainable goal on any basis except by laboriously **isolating** a specific complex, identifying the location of the epitope, and sequencing that epitope. The problem is further complicated by the. . .

SUMM . . . (1988, 14th International Congress of Biochemistry, Volume 5, Abstract FR:013) described a method to produce a mixture of peptides by **separately** coupling each of three different amino acids, then mixing all of the resin. The procedure described by Furka et al. provides no satisfactory method to **isolate** a peptide of

interest from the plurality of peptides produced.

SUMM . . . a single solid phase support. The representation of only one species on a support would greatly enhance current techniques for **isolating** peptides.

SUMM . . . random peptide sequences, and oligonucleotide sequences, i.e., bio-oligomer sequences in which a single bio-oligomer species can be readily and quickly **isolated** from the rest of the library. There is also a need in the art for a method for quickly and. . .

SUMM . . . method for generating the library comprising repeating the steps of providing at least two aliquots of a solid phase support; **separately** introducing a set of subunits to the aliquots of the solid phase support; completely coupling the subunit to substantially all. . .

SUMM . . . said substrate molecule will undergo a chemical reaction catalyzed by one or more solid phase support/bio-oligomer species within the library; **isolating** a solid phase support/bio-oligomer combination that exhibits the desired property; and sequencing the bio-oligomer of the **isolated** solid phase support/bio-oligomer. In a different embodiment, a portion of the bio-oligomer is released from the solid phase support/bio-oligomer combination. . .

DETD (ii) **separately** introducing a set of subunits to the aliquots of the solid phase support;

DETD A set of first amino acids is **separately** introduced to each aliquot. Generally, the amino acids used for peptide synthesis are the base-labile N.sup..alpha. -amino protected 9-fluorenylmethoxycarbonyl (Fmoc). . .

DETD To each aliquot is **separately** introduced a second set of amino acids. This second set may consist of (a) the same amino acids added in.

. .

DETD . . . cycle and that the coupling is driven to completion. The one bead-one peptide synthesis allows increased sensitivity and efficiency of **isolating** the peptide that is specific for the entity to which is binds.

DETD . . . acid to form a lactone or a lactam, and a chelator such as .gamma.-carboxyl-glutamic acid (Gla) (Bachem) to chelate a **transition metal** and form a cross-link. Protected .gamma.-carboxyl glutamic acid may be prepared by modifying the synthesis described by Zee-Cheng and Olson. . .

DETD . . . deoxynucleoside could be fixed, for example, as deoxyadenosine. After detritylation, and washing with dichloromethane followed by acetonitrile, the solid-support is **separated** into four equal aliquots and transferred into four **separate** reaction vessels. The four deoxynucleoside 3'-phosphoramidites are then added individually into the four **separate** reaction vessels. After the completion of coupling the solid-supports from the four reaction vessels are mixed together, thoroughly washed and. . .

DETD . . . POLYHIPEO resin (obtained from Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene resin grafted with polyethylene glycol (TentaGels, Rapp **Polymere**, Tubingen, Germany) or **polydimethylacrylamide** resin (obtained from Milligen/Biosearch, California). In a preferred embodiment for peptide synthesis, solid phase support refers to polydimethylacrylamide resin.

DETD . . . acceptor molecules. By identifying the particular bio-oligomer species to which a specific acceptor molecule binds, it is possible to physically **isolate** the bio-oligomer species of interest.

DETD Because only a small number of beads will be removed during each screening/detection/**isolation** step, the majority of the beads will remain in the pool. Therefore, the random bio-oligomer library can be reused multiple. . .

DETD . . . found on a single solid phase support so that the support, and thus the bio-oligomer, can be readily identified and **isolated**.

DETD The bio-oligomer can be **isolated** by any conventional means

known to those of ordinary skill in the art and the invention is not limited by the method of **isolation**. For example and not by way of limitation, it is possible to physically **isolate** a solid phase support/bio-oligomer combination that exhibits the strongest physico-chemical interaction with the specific acceptor molecule. In one

embodiment based. . . the peptide and antibody, for example, one hour

at 22.degree. C. Thereafter, the acceptor molecule coated bio-oligomer/solid phase support is **isolated**. More specific embodiments are set forth in the following methods, which describe the use of a monoclonal antibody as a. . .

DETD . . . C. The magnetic beads will form a rosette around the solid phase support/peptide of interest which can then be physically **isolated** with a strong magnet.

DETD . . . (becomes blue) due to precipitation of the converted substrate on the solid phase support, and can be easily identified and **isolated** physically under a dissecting microscope with a micromanipulator. The relative intensity of the color reaction is generally proportional to the. . .

DETD . . . or 4-chloro-1-napthol (4CN). After incubating for several minutes, the antibody-solid phase support/peptide combination changes color, and can be identified and **isolated** physically under a dissecting microscope with a micromanipulator. The relative intensity of

the color reaction is generally proportional to the. . .

DETD . . . color is developed as described above in (iii) with the enzyme method. The peptide/solid phase support of interest is physically **isolated** as above.

DETD . . . library to form a "rosette" between the target cells and the relevant solid phase support/peptide. The rosette can thereafter be **isolated** by differential centrifugation or removed physically under a dissecting microscope.

DETD . . . be performed with two or more colored latex beads, or fluorophores that emit at different wavelengths. Labeled beads may be **isolated** manually or by mechanical means. Mechanical means include fluorescence activated sorting, i.e., analogous to FACS, and micromanipulator removal means.

DETD Reactive beads may be **isolated** on the basis of intensity of label, e.g., color intensity, fluorescence intensity, magnetic strength,

or radioactivity, to mention a few. . .

DETD . . . which it was released. Thus, evidence of biological activity of

interest, in proximity to a bead, will allow identification and **isolation** of the bead, and sequencing or other characterization of the bio-oligomer. Identification of the bio-oligomer is possible because enough will. . .

DETD . . . to release another 1/3 of bio-oligomer, and the supernatant assayed for biological activity. Beads from wells demonstrating biological activity are **isolated** and the attached bio-oligomer is sequenced. Where more than one bead is found, all the identified sequences are prepared and. . .

DETD . . . may then be screened. In one aspect, a library may be introduced into an animal. Beads of interest may be **isolated** from a specific tissue. Beads may be **isolated** that were specifically absorbed after oral, nasal, or cutaneous administration. In

a preferred embodiment, such beads are magnetic, or have some other identifying feature, and thus are readily **isolated** from the tissue.

DETD . . . is determined by nonlinear regression analysis using the logistic equation. Data from both standard curves will be analyzed together and **separately** to determine if there is a significant difference in the response measured at both ends of the bioassay procedure (F-ratio. . .

DETD The peptides can be sequenced either attached to or cleaved from the solid support. To cleave the peptide, the **isolated** peptide-beads are treated with traditional cleaving agents known to those of skill in this art to **separate** the polymer from the solid phase supports. The choice of cleaving agent selected will depend on the solid phase support. . .

DETD Alternatively, in another embodiment within the scope of the invention, it is possible to **isolate** a single solid phase support, such as a bead, with its attached bio-oligomers and apply the bead to a sequencer. . .

DETD . . . that a few of the millions of bio-oligomers in the pool may provide sequences that have biological activity, one may **isolate** bio-oligomers that possess antitumor, anti-animal parasite, or antimicrobial, e.g., antifungal, antibacterial, anti-unicellular parasite, anti-unicellular pathogen, or antiviral activities. In addition. . .

DETD Peptides that bind to tumor-specific monoclonal antibodies could be **isolated**, sequenced and synthesized for use as an immunogen to induce active immunity against the tumor.

DETD . . . of DCM and DMF (2 times), the resin was resuspended in approximately 7.5 ml of DMF, and divided into three **separate** aliquots of approximately 2.5 ml each and distributed into three numbered coupling tubes.

DETD . . . washing with DCM and DMF as described above, the mixture was divided into three aliquots and distributed into the three **separate** reaction vessels. A second set of amino acids was added. After coupling was complete, the resin was first deprotected with. . .

DETD **ISOLATION** OF A PEPTIDE LIGAND THAT BINDS TO A RECEPTOR MOLECULE

DETD To demonstrate the use of the method of the instant invention to **isolate** a particular peptide, a 12 amino acid peptide with the predetermined sequence from the V-mos gene product was synthesized. V-mos is an oncogene **isolated** from mouse sarcoma, and is related to the Moloney murine sarcoma virus. The v-mos gene product is known to have. . .

DETD . . . in a lawn of colorless beads. Accordingly, the beads that contained the peptide sequence of interest were readily distinguished and **isolated** from the other beads in the library.

DETD After **isolation**, the Applied Biosystems 477A Protein Sequencer was employed to determine the N-terminal amino acid sequences of a single "long v-mos". . .

DETD . . . interest from among a thousand-fold excess of non-binding, irrelevant beads. Furthermore, this Example demonstrates that a reactive bead may be **isolated** and the sequence of the peptide determined.

DETD **ISOLATION** OF A SHORTER PEPTIDE LIGAND THAT BINDS TO A RECEPTOR MOLECULE

DETD To further demonstrate the use of the method of the instant invention to **isolate** a particular peptide, a hexapeptide with the predetermined sequence Gly-Phe-Gly-Ser-Val-Tyr was synthesized on the standard 100 .mu.m PAM resin using. . .

DETD . . . rather is specific for a solid phase support/peptide combination. As in Example 8, supra, a positively reacting bead may be **isolated**, and the attached peptide sequenced.

DETD . . . of huge peptide libraries with each different peptide on an individual bead. Individual specific binding peptide beads are then physically **isolated** on the bead and the sequence of the attached peptide determined.

DETD . . . depends on the ability to chemically synthesize a huge random peptide library and to couple it to an appropriate detection **isolation**, and structure determination system.

DETD The means of eliminating this problem provided by the present invention is to **separate** the resin beads into a series of individual

equal aliquots during each coupling cycle, and to allow each aliquot of.

. . individual activated amino acid. After complete coupling, the various aliquots of resin are thoroughly mixed, washed, deprotected, washed, and again **separated** into aliquots for a new cycle of coupling. Accordingly, no one resin bead is exposed to more than one amino. . .

DETD . . . linker. Randomization was carried out in the next five coupling steps, and all 19 Fmoc-amino acid-OPfp except cysteine were used **separately** during each coupling step. After the five coupling steps were completed, the Fmoc group was removed in 20% piperidine (v/v). . .

DETD . . . contains a single peptide species. An individual resin bead that interacts with an acceptor molecule can then be identified, physically **isolated**, and the amino acid sequence of the peptide ligan will then be determined by Edman degradation. The success of the. . .

DETD . . . specific peptide ligands from the random library can easily be accomplished with immunological techniques, such as an Enzyme Linked Immunoabsorbant **Assay** (**ELISA**), immunofluorescence or with immunomagnetic beads. For the experiments described herein, immunohistochemical techniques were used in the detection system. The specific-binding. . .

DETD Peptide ligand binding affinities for the anti-.beta.-endorphin monoclonal antibody were determined in solution phase. The anti-.beta.-endorphin binding **assay** measured peptide ligand inhibition of 5.0 nM [.sup.3 H][Leu]enkephalin (specific activity=39.0 Ci/mmole, New England Nuclear, Boston, Mass.) binding to 125-200. . .

DETD . . . 16 hours. After extensive washing, a secondary step with streptavidin-alkaline phosphatase was used to trigger the staining reaction for the **ELISA**. Six peptides with consensus sequence that have close resemblance to the native ligand, Leu-enkaphalin (YGGFL), were identified in this screening:. . . [.sup.3 H]Leu-enkaphalin (New England Nuclear, Boston, Mass.) as the labelled ligand and the unlabelled peptides as the competing ligand (anti-.beta.-endorphin **assay**, Section 10.1.2, supra). The results of these studies are summarized in Table 2.

DETD . . . Science 259:404-406, Section 2., supra) reported the importance of HPQ, HPM, and HPN sequences in their 20 streptavidin binding ligands **isolated** with the fusion filamentous phage technique. Of their 20 **isolates**, 15 had HPQ, 4 had HPM, and 1 had HPN consensus sequences. Interestingly, the peptide library yielded 28 different peptides,. . . in sharp contrast to the data reported by Devlin et al., supra, where there were multiple repeats among their 20 **isolates** suggesting that selection bias occurred in their biosynthetic method.

DETD . . . equimolar amount of [.sup.3 H]sodium acetate (specific activity=2.52 Ci/mmole, New England Nuclear, Boston, Mass.). The [.sup.3 H]Ac-v-mos product, which was **separated** from unreacted v-mos peptide with reverse phase HPLC, had a specific activity of 2.50 Ci/mmole. The binding affinity of [.sup.3. . . in-the presence (nonspecific) and absence (total) of 100 .mu.M unlabelled v-mos peptide for each [.sup.3 H]Ac-v-mos concentration. Bound radioligand was **separated** by centrifugation using a 10-fold excess (binding capacity relative to immunoglobulin used) of Protein-G Sepharose (Pharmacia) to precipitate the antibody.. . .

DETD Either (1) a portion or the whole amount of medium is transferred into a **separate** plate with a specific cancer cell line, or (2) the cancer cell line is added directly into the well with. . .

CLM What is claimed is:

. . . that said acceptor molecule will recognize and bind to one or more solid phase support/bio-oligomer species within the library; (b)

isolating a solid phase support/bio-oligomer combination that exhibits binding with the acceptor molecule; and (c) determining the chemical structure of the bio-oligomer of the solid phase support/bio-oligomer **isolated** in step (b).

. . . of a non-cleavable linker and a selectively cleavable linker, by means of which the bio-oligomer can be attached covalently; (ii) **separately** introducing a species of subunits of the bio-oligomers to each of the aliquots of solid phase supports such that a. . . . support/bio-oligomer by cleavage of a clearable linker; (c) detecting the biological activity of the released bio-oligomer interest in situ; (d) **isolating** a solid phase support/bio-oligomer combination that exhibits the specific biological activity of interest; and (e) determining the chemical structure of the bio-oligomer remaining on the solid phase support/bio-oligomer **isolated** in step (d).

. . . the solid phase support/bio-oligomer combination in situ; (c) detecting inhibition of the enzyme catalyzed reaction of interest in situ; (d) **isolating** a solid phase support/bio-oligomer combination detected in step (c); (e) determining the chemical structure of the bio-oligomer remaining on the solid phase support/bio-oligomer **isolated** in step (d).

. . . said supports having a reactive site to which a species of subunit of the bio-oligomer can be covalently coupled; (ii) **separately** introducing a species of subunits of the bio-oligomers to each of the aliquots of solid phase supports such that a. . . that said acceptor molecule will recognize and bind to one or more solid phase support/bio-oligomer species within the library; (b) **isolating** a solid phase support/bio-oligomer combination that exhibits binding with the acceptor molecule; and (c) determining the chemical structure of the bio-oligomer of the solid phase support/bio-oligomer **isolated** in step (b).

L10 ANSWER 40 OF 55 USPATFULL
ACCESSION NUMBER: 95:92727 USPATFULL
TITLE: Ion-**capture assays** using a specific binding member conjugated to carboxymethylamylose
INVENTOR(S): Adamczyk, Janina, Gurnee, IL, United States
Berry, Daniel S., Libertyville, IL, United States
Jou, Yi-Her, Libertyville, IL, United States
Stroupe, Stephen D., Libertyville, IL, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5459080	19951017
APPLICATION INFO.:	US 1994-187814	19940127 (8)

DISCLAIMER DATE: 20140127
RELATED APPLN. INFO.: Continuation of Ser. No. US 1991-707726, filed on 30 May 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-375029, filed on 7 Jul 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-150278, filed on 29 Jan 1988, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Scheiner, Toni R.
ASSISTANT EXAMINER: Parsons, Nancy J.
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
LINE COUNT: 3264
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention includes novel **assays** employing a **capture** reagent, involving a first specific binding member conjugated to a polymeric anion such as carboxymethylamylose, and a solid phase material containing a reaction site comprising a polymeric cation substance. A test sample suspected of containing the analyte of interest may be contacted with the **capture** reagent to form a charged **capture** reagent/analyte complex. The complex is then contacted to/ the oppositely charged solid phase to attract, attach, and immobilize the **capture** reagent/analyte complex. The use of carboxymethylamylose to prepare a suitably charged **capture** reagent provides a superior **capture** reagent that is capable of binding and retaining the analyte on the solid phase even in the presence of polyanionic non-specific binding blockers.

TI Ion-**capture assays** using a specific binding member conjugated to carboxymethylamylose

AB The present invention includes novel **assays** employing a **capture** reagent, involving a first specific binding member conjugated to a polymeric anion such as carboxymethylamylose, and a solid phase material. . . comprising a polymeric cation substance. A test sample suspected of containing the analyte of interest may be contacted with the **capture** reagent to form a charged **capture** reagent/analyte complex. The complex is then contacted to/ the oppositely charged solid phase to attract, attach, and immobilize the **capture** reagent/analyte complex. The use of carboxymethylamylose to prepare a suitably charged **capture** reagent provides a superior **capture** reagent that is capable of binding and retaining the analyte on the solid phase even in the presence of polyanionic. . .

SUMM This invention relates generally to the field of binding **assay** devices and methods. In particular, the present invention relates to novel devices useful in the performance of homogeneous **immunoassays**.

SUMM Various analytical procedures and devices are commonly employed in **assays** to determine the presence and/or concentration of substances of interest or clinical significance which may be present in biological liquids. . .

SUMM **Immunoassay** techniques take advantage of the mechanisms of the immune systems of higher organisms, wherein antibodies are produced in response to. . .

SUMM There are several known **immunoassay** methods using immunoreactants, wherein at least one of the immunoreactants is labeled with a detectable component so as to be. . . one labeled antibody and an unlabeled antibody bound to a solid phase support such that the complex can readily be **isolated**. In this example, the amount of labeled antibody associated with the solid phase is directly proportional to the amount of. . .

SUMM An alternative technique is the "competitive" **assay**. In one example of a competitive **assay**, the **capture** mechanism again may use an antibody attached to an insoluble solid phase, but a labeled analyte (rather than a labeled. . . immobilized antibody. Similarly, an immobilized analyte can compete with the analyte of interest for a labeled antibody. In these competitive **assays**, the quantity of **captured** labeled reagent is inversely proportional to the amount of analyte present in the sample.

SUMM Despite their great utility, there are disadvantages with such **assay** methods. First, the heterogenous reaction mixture of liquid test sample and soluble and insoluble **assay** reagents, can retard the kinetics of the reaction. In comparison to a liquid phase reaction wherein all reagents are soluble,. . . referred to as nonspecific binding and can interfere with the detection of a positive result. Third, with conventional immobilization methods, **separate** batches of manufactured solid phase reagents can contain variable amounts of immobilized binding member.

SUMM With regard to the manufacture of solid phase devices for use in binding **assays**, there are a number of **assay** devices and procedures wherein the presence of an analyte is indicated by the analyte's binding to a labeled reagent and/or. . .

SUMM The use of porous test strips in the performance of specific binding **assays** is also well-known. In a sandwich **assay** procedure, a test sample is applied to one portion of the test strip and is allowed to migrate through the. . . a component of the fluid test sample or with the aid of an eluting or chromatographic solvent which can be **separately** added to the strip. The analyte is thereby transported into a detection zone on the test strip wherein an analyte-specific. . . aid of a labeled analyte-specific binding member which may be incorporated in the test strip or which may be applied **separately** to the strip.

SUMM . . . a solution by capillary action. Different areas or zones in the strip contain the reagents needed to perform a binding **assay** and to produce a detectable signal as the analyte is transported to or through such zones. The device is suited for chemical **assays** as well as binding **assays** which are typified by the binding reaction between an antigen and a complementary antibody.

SUMM . . . an immunosorbing zone, containing an immobilized specific binding member. The test sample is applied to the immunosorbing zone, and the **assay** result is read at the immunosorbing zone.

SUMM Alternative **separation** methods include the use of a magnetic solid phase, polymerization techniques and the formation of analyte

complexes having characteristics different than the non-complexed analyte. Ullman et al. (U.S. Pat. No. 4,935,147) describe a method for **separating** charged suspended non-magnetic particles from a liquid medium by contacting the particles with charged magnetic particles and a chemical reagent.. . .

SUMM Longoria et al. (U.S. Pat. No. 4,948,726) describe an **assay** method involving the reaction of antigen and antibody molecules to form an antigen/antibody complex that uniquely exhibits an ionic charge. . . is then chosen for its unique affinity for the antigen/antibody complex. Milburn et al. (U.S. Pat. No. 4,959,303) describe an **assay** wherein antigen from a test sample and an antibody specific for the antigen are incubated under conditions sufficient for the. . .

SUMM Vandekerckhove (U.S. Pat. No. 4,839,231) describes a two-stage, protein immobilization process involving an initial **separation** or **isolation** of target proteins in a gel, such as a polyacrylamide electrophoresis gel, followed by the transfer of those **isolated** proteins to the surface of a coated support for immobilization. The coated support is prepared by contacting a chemically inert. . .

SUMM . . . strip field. There is a growing demand for devices that require few or no manipulative steps to perform the desired **assay**, for devices that can be used by relatively untrained personnel, and for devices that provide results which are minimally affected by variations in the manner in which the **assay** is performed. Further considerations are the ease with which the resultant detection signal may be observed as well as the. . . addition, a device manufacturing format has long been sought which will enable the production of a "generic" device, i.e., an **assay** device for which the capacity of use is defined by the reagents used in the performance of the **assay** rather than the reagents used in the manufacture of the device.

SUMM The present invention provides novel binding **assay** methods for determining the presence or amount of an analyte in a test sample. The **assay** involves a **capture** reagent, containing a first binding member conjugated to a polymeric anion such as carboxymethylamylose, an indicator reagent containing a second. . . a solid phase material containing a reaction site made of a polymeric cation substance. The specific binding members of the **capture** reagent and indicator are chosen for the formation of a complex with the analyte in a sandwich **assay**, a competitive **assay** or an indirect **assay**, thereby forming a detectable complex in proportion to the presence or amount of the analyte in the test sample.

SUMM The solid phase is contacted with the **capture** reagent and the test sample, whereby the polymeric cation of the solid phase attracts and attaches to the polymeric anion of the **capture** reagent, thereby immobilizing the **capture** reagent and complexes thereof upon the solid phase. The solid phase may then be contacted with the indicator reagent, whereby the indicator reagent becomes bound to the immobilized **capture** reagent, or complex thereof, in proportion to the amount of analyte present in the test sample. Typically, the indicator reagent. . .

SUMM The present invention also enables the production of a generic solid phase device for use in specific binding **assays**. **Assay** procedures for many different analytes can use the same solid phase material which contains a predetermined zone of anionic or cationic **capture** polymer rather than an immobilized binding member capable of binding only a specific analyte as found in conventional flow-through and. . .

SUMM The specific binding member component of the **capture** reagent can be either a hapten or a macromolecule. The charged **capture** reagent enables homogeneous **assay** and **separation** reactions wherein the reaction complexes can be removed from the reaction mixture by contacting the mixture with an oppositely charged

solid phase. Virtually any binding **assay** (sandwich **assays**, competitive **assays**, indirect **assays**, **assays** using ancillary specific binding members, inhibition **assays**, etc.) can be adapted to use the novel **capture** reagents and ion-**capture** techniques of the present invention.

SUMM The present invention provides two major advancements to the field of specific binding **assays**: a) the use of liquid phase kinetics facilitates the formation of a complex from the homogeneous mixture of analyte and **assay** reagent specific binding members, and b) the ion-**capture** technique increases the potential number of complexes that can be immobilized on a solid support. If the advantages of liquid. . .

SUMM The novel **capture** reagent of the present invention can also be used in a **separation** procedure. A liquid sample containing an analyte to be **separated** from the sample is mixed with the **capture** reagent and reacted to form a charged analyte/**capture** reagent complex. Following the specific binding reaction, the solution is contacted to an oppositely charged solid phase which attracts, attaches to, and **separates** the newly formed complex from the liquid sample.

DETD The **assay** methods and reagents of the present invention can be used in a variety of **immunoassay** formats. The present invention, however, is not limited to immunoreactive **assays**. Any **assays** using specific binding reactions between the analyte and **assay** reagents can be performed.

DETD . . . specific binding pairs are exemplified by the following: biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences (including probe and **capture** nucleic acid sequences used in DNA hybridization **assays** to detect a target nucleic acid sequence), complementary peptide sequences (including those formed by recombinant methods), effector and receptor molecules,. . .

DETD . . . pretreated prior to use, such as preparing plasma from blood, diluting viscous liquids, etc. Methods of pretreatment can also involve **separation**, filtration, distillation, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples such as. . .

DETD The term "analyte", as used herein, refers to the substance to be detected in or **separated** from the test sample by means of the present invention. The analyte can be any substance for which there exists. . .

DETD The term "signal producing component", as used herein, refers to any substance capable of reacting with the analyte or another **assay** reagent to produce a reaction product or signal that indicates the presence or amount of the analyte and that is detectable by visual or instrumental means. "Signal production system", as used herein, refers to the group of **assay** reagents that are used to produce the desired reaction product or signal. For example, one or more signal producing components. . .

DETD . . . the amount of an analyte in the test sample. Generally, the indicator reagent is detected or measured after it is **captured** on the solid phase material, but the unbound indicator reagent can also be measured to determine the result of an **assay**.

DETD The specific binding member of the indicator reagent is capable of binding either to the analyte as in a sandwich **assay**, to the **capture** reagent as in a competitive **assay**, or to an ancillary specific binding member to complete a detectable complex. The label, as described above, enables the indicator. . . reagent enables the indirect binding of the label to the analyte, to an ancillary specific binding member or to the **capture** reagent. The selection of a particular label is not critical, but the label will be capable of generating a detectable. . .

DETD As mentioned above, the label can become attached to the specific binding member during the course of the **assay**. For example, a

biotinylated anti-analyte antibody may be reacted with a labeled streptavidin molecule. Any suitable combination of binding members. . .

DETD The term "**capture** reagent", as used herein, refers to an unlabeled specific binding member which is attached to a charged substance. The attachment. . . attachment to the charged substance does not interfere with the binding member's binding site. The binding member component of the **capture** reagent is specific either for the analyte as in a sandwich **assay**, for the indicator reagent or analyte as in a competitive **assay**, or for an ancillary specific binding member, which itself is specific for the analyte.

DETD The charged substance component of the **capture** reagent can include anionic and cationic monomers or polymers. For example, anionic polymers include polyglutamic acid (PGA), anionic protein or. . . member can be joined to more than one charged monomer or polymer to increase the net charge associated with the **capture** reagent.

DETD The novel **capture** reagents of the present invention are used to facilitate the observation of the detectable signal by substantially **separating** the analyte and/or the indicator reagent from other **assay** reagents and the remaining test sample components. In its most advantageous use, the **capture** reagent is reacted with the test sample and **assay** reagents in a homogeneous reaction mixture. Following the formation of the desired specific binding member complexes, the complexes involving a **capture** reagent are removed from the homogeneous reaction mixture by contacting the homogeneous reaction mixture to a solid phase that is oppositely charged with respect to the charge of the **capture** reagent.

DETD In one embodiment of the present invention, a negatively charged **capture** reagent can be prepared by conjugating the selected specific binding member to one or more activated polymeric anionic molecules and. . .

DETD Typically, the negatively charged **capture** reagents of the following Examples were formed by reacting the desired specific binding member with an activated PGA molecule having. . .

DETD . . . used to "activate" a specific binding member or polymeric anionic molecule, i.e., to prepare the specific binding member or the **polymeric** anionic molecule for chemical **cross-linking**. Activating agents also include thiol introducing agents such as the thiolanes (such as 2-iminothiolane), succinimidyl mercaptoacetates (such as N-succinimidyI-S-acetylmercaptoacetate), and. . .

DETD . . . preferred agents for use with the particular polymeric anionic molecule and specific binding member to be used in the diagnostic **assay**. Therefore, it will be appreciated by those skilled-in-the-art that the coupling agent or activating agent used in a given **assay** will generally be determined empirically.

DETD . . . a sulfur stabilizer and R" is an aliphatic or aryl group. An example of the preparation of a negatively charged **capture** reagent involves the reaction of a specific binding member (SBM) having an amino group and an activated PGA having an. . .

DETD In yet another embodiment of the present invention, a preferred anionic polymer for use in the **capture** reagent is carboxymethylamylose (CMA) due to its particular performance in various **immunoassay** configurations. The improved performance of **capture** reagents containing CMA can be attributed to the higher avidity of the CMA **capture** reagent for the cationic solid phase. This attribute is particularly advantageous in a two step sandwich **assay** format wherein a polyanion is used to block nonspecific binding of the indicator reagent to the cationic solid phase.

DETD . . . specific binding member", as used herein, refers to any member of a specific binding pair which is used in the **assay** in addition to the specific binding members of the **capture** reagent and the indicator reagent. For example, in an **assay** an ancillary specific binding member may bind the analyte to a second

specific binding member to which the analyte itself could not attach, or
as in an inhibition **assay** the ancillary specific binding member may be a reference binding member. One or more ancillary specific
binding members can be used in an **assay**.

DETD . . . insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic charge and ability to attract the **capture** reagent, e.g., methylated wool, nylons, and special glasses having a positive charge. Alternatively, the solid phase can be pretreated with and retain a charged substance that is oppositely charged with respect to the charged substance of the **capture** reagent. For example, an anionic substance can be bound to a specific binding member to form the **capture** reagent, and a cationic substance can be applied to and retained by the solid phase, or vice versa.

DETD . . . about 2% (exclusive of counter ion) are particularly advantageous in preparing a solid phase that will undergo washing during
the **assay** process. The use of such a polycationic substance to prepare a suitably charged solid phase resulted in a solid phase. . . could be subjected to a greater degree of manipulation without losing the capability to attract and retain the oppositely charged **capture** reagent. It was determined that polycationic substances having a nitrogen content above about 5% (exclusive of counter ion) were
more. . .

DETD An **assay** device based on the ion-**capture** technique can have many configurations, several of which are dependent upon the material chosen as the solid phase. In various. . .

DETD The novel ion-**capture** devices of the present invention involve a solid phase made of any suitable porous material. By "porous" is meant
that. . . phase materials. For example, the solid phase can include a
fiberglass, cellulose, or nylon pad for use in a flow-through **assay** device having one or more layers containing one or more of the **assay** reagents; a dipstick for a dip and read **assay**; a test strip for wicking or capillary action (e.g., paper, nitrocellulose, polyethylene) techniques; or other porous or open
pore materials. . .

DETD . . . cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon); porous gels such as silica gel, agarose, dextran, and gelatin; **polymeric** films such as **polyacrylamide**; and the like. The solid phase should have reasonable strength or strength can be provided by means of a support,. . .

DETD Preferred solid phase materials for flow-through **assay** devices include filter paper such as a porous fiberglass material or other fiber
matrix materials as well as isotropically porous. . . the material used will be a matter of choice, largely based upon the properties of the sample or analyte being **assayed**, e.g., the fluidity of the test sample.

DETD Typically, the novel test strip and flow-through devices employing the ion-**capture** principles of the present invention are characterized by having the analyte, test sample and/or eluting solvent migrate through the device. . .

DETD Uses for Ion-**Capture** Reagents

DETD In accordance with the disclosure of the present invention, a sandwich **assay** can be performed wherein the **capture** reagent involves an analyte-specific binding member which has been bound to a charged substance such as an anionic polymer. The **capture** reagent is contacted with a test sample, suspected of containing the analyte, and an indicator reagent comprising a labeled analyte-specific.

. .

DETD In the exemplary sandwich **assay**, a binding reaction results in the formation of a **capture** reagent/analyte/indicator reagent complex. The resultant complex is then removed from the excess **assay** reagents and test sample of the homogenous reaction mixture by means of a solid phase that is either inherently oppositely charged with respect to the **capture** reagent or that retains an oppositely charged substance, for example a cationic polymer. In the ion-**capture assays**, the oppositely charged solid phase attracts and attaches to the **capture** reagent/analyte/indicator reagent complex through the interaction of the anionic and cationic polymers. The complex retained on the solid phase is. . . solid phase is proportional to the amount of analyte in the sample. The only major limitation inherent in the sandwich **assay** is the requirement for the analyte to have a sufficient size and appropriately orientated epitopes to permit the binding of at least two specific binding members. Other sandwich **assays** may involve one or more ancillary specific binding members to bind the analyte to the indicator reagent and/or **capture** reagent.

DETD The present invention also can be used to conduct a competitive **assay**. In an exemplary competitive **assay**, the soluble **capture** reagent again includes a specific binding member which has been attached to a charged substance, such as an anionic polymer. The **capture** reagent is contacted, either sequentially or simultaneously, with the test sample and an indicator reagent that includes a second binding member which has been labeled with a signal generating compound. Either the **capture** reagent and analyte can compete in binding to the indicator reagent (e.g., the **capture** reagent and analyte are antigens competing for a labeled antibody), or the indicator reagent and analyte can compete in binding to the **capture** reagent (e.g., the indicator reagent is a labeled antigen which competes with the antigen analyte for binding to the antibody component of the **capture** reagent). A competitive binding or displacement reaction occurs in the homogeneous mixture and results in the formation of **capture** reagent/analyte complexes and **capture** reagent/indicator reagent complexes.

DETD The resultant complexes are removed from the excess **assay** reagents and test sample by contacting the reaction mixture with the oppositely charged solid phase. The **capture** reagent complexes are retained on the solid phase through the interaction of the oppositely charged polymers. The complexes retained on the solid phase can be detected via the label of the indicator reagent. In the competitive **assay**, the amount of label that becomes associated with the solid phase is inversely proportional to the amount of analyte in the sample. Thus, a positive test sample will generate a negative signal. The competitive **assay** is advantageously used to determine the presence of small molecule analytes, such as small peptides or haptens, which have a single epitope with which to bind a specific binding partner. Other competitive **assays** may involve one or more ancillary specific binding members to bind the analyte to the indicator reagent and/or **capture** reagent.

DETD For example, in an **assay** for theophylline, an anti-theophylline antibody (either monoclonal or polycional) can be conjugated with an anionic polymer to form a soluble **capture** reagent, and a competition for binding to that antibody can be established between labeled theophylline (i.e., indicator reagent) and the. . . to a solid phase which retains a cationic polymer coating. The attraction between the oppositely charged ionic species of the **capture** reagent and the solid phase serves to **separate** the immunocomplex from the reaction mixture. The signal from the indicator reagent can then be detected. In this example, increased. . .

DETD In addition, the present invention can be used in an inhibition **assay**, such as the measurement of an antibody by inhibiting the detection of a reference antigen. For example, the **capture**

reagent can include an antibody/anionic polymer conjugate and the indicator reagent can be a labeled antibody. The test sample, suspected of containing an antibody analyte, is mixed with a reference antigen with which the **capture** reagent and indicator reagent can form a detectable sandwich complex that can be immobilized upon the solid phase by the ion-**capture** reaction. The degree of inhibition of antigen uptake by the **capture** reagent is proportional to the amount of antibody analyte in the test sample, thus, as the concentration of the antibody. . .

DETD In general, once complex formation occurs between the analyte and the **assay** reagents, the oppositely charged solid phase is used as a **separation** mechanism: the homogeneous reaction mixture is contacted with the solid phase, and the newly formed binding complexes are retained on the solid phase through the interaction of the opposite charges of the solid phase and the **capture** reagent. If the user is not concerned with liquid phase kinetics, the **capture** reagent can be pre-immobilized on the solid phase to form a **capture** site.

DETD The present invention can also be used for **separating** a substance from a liquid sample. For example, the **capture** reagent and solid phase can be used without an indicator reagent for the sole purpose of **separating** an analyte from a test sample. Furthermore, the **capture** reagent can be contacted with a soluble second charged substance which is oppositely charged with respect to the **capture** reagent. The second charged substance is not retained on the solid phase prior to contacting the sample to the solid phase material, but it attracts and attaches to the **capture** reagent such that the resultant **assay** complexes are retained on an oppositely charged solid phase.

DETD When the complex of charged **capture** reagent and analyte (and/or indicator reagent) is contacted to the oppositely charged solid phase, the ionic attraction of the oppositely charged species governs the efficiency of the **separation** of the complex from the reaction mixture. The ionic attraction can be selected to provide a greater attraction than the immunological attraction of antibody for antigen, particularly when multiple polycationic and polyanionic species are included in the **capture** reagent and oppositely charged solid phase. A further advantage is that the "ion-**capture**" technique minimizes the nonspecific adsorption of interfering substances onto the solid phase, thereby offering improved accuracy of analysis. The ion-**capture** technique thereby enables the performance of an **assay** having a highly specific **separation** method, minimal nonspecific binding, and high sensitivity.

DETD . . . to noise ratio. It was unexpectedly discovered that the nonspecific binding blocker could be a free polyanion even when the **capture** reagent used in the **assay** involved a polyanionic substance conjugated to a specific binding member. It would have been expected that the presence of a free or unbound polyanion would prevent, or at least reduce, the immobilization of the **capture** reagent on the solid phase. It was found, however, that the nonspecific blocker was more effective in inhibiting the direct, nonspecific binding of indicator reagent to the solid phase than it was in reducing the attachment of the polyanionic **capture** reagent to the polycationic solid phase. Suitable nonspecific binding blockers include, but are not limited to, dextran sulfate, heparin, carboxymethyl. . .

DETD . . . nonspecific binding blocker added to the indicator reagent could be greater than the amount of polyanionic substance contained in the **capture** reagent. It was found that free polyanionic nonspecific binding blocker could be added to the indicator reagent in amounts 40,000 times the amount of polyanionic substance used in the **capture** reagent. Generally, the preferred amount of polyanionic

blocker added to the indicator reagent is 50 to 14,000 times the amount of polyanionic substance used in the **capture** reagent. For two step sandwich **assays**, the preferred amount of polyanionic blocker added to the indicator reagent is 1000 to 2000 times that contained in the **capture** reagent.

DETD An appropriate range of use can be determined for each analyte of interest. For example, in an **assay** to detect thyroid stimulating hormone (TSH) wherein dextran sulfate was added to the indicator reagent as a free polyanionic nonspecific binding blocker, suitable amounts of free polyanion ranged from 233 to 19,000 times that of the **capture** reagent, or about 0.1-8% dextran sulfate. As illustrated in the following Table, the preferred nonspecific binding blocker as well as. . .

DETD

Nonspecific Binding Blocker in the Indicator Reagent Analyte	Preferred	More Preferred
	% Dextran sulfate (MW 5,000) (blocker/**capture** reagent, w/w)	
TSH	0.1-8 (233-19,000)	0.5-2 (1,000-4,000)
T3	0.1-2 (2,000-40,000)	0.1-0.2 (2,000-4,000)
	% Carboxymethyl cellulose (MW 250,000) (blocker/ **capture** reagent, w/w)	
hCG	0.01-0.25 (0.44-11)	0.025 (1.1)
HIV	0-0.2 (0-20,000)	0.05 (5,000)

DETD Moreover, it was discovered that the polyanionic nonspecific binding blocker could be added to the **assay** as a **separate** reagent, or it could be included as free polyanion in the **capture** reagent, in an ancillary binding member reagent, in a buffer reagent or in some other reagent used in the **assay**. For example, when free polyanion is included in the **capture** reagent, it can enhance the signal to noise ratio by neutralizing interfering materials which are contained either in the test sample itself or in the other **assay** reagents, or those which were introduced during the device manufacturing process. The following Table illustrates some preferred amounts of nonspecific binding blocker for different analytes of interest, wherein the free polyanion is contained in the **capture** reagent itself.

DETD

Nonspecific Binding Blocker in the **Capture** Reagent Analyte	Preferred	More Preferred
	% Dextran sulfate (MW 5,000) (blocker/**capture** reagent, w/w)	
Digoxin	0-0.004 (0-222)	0.004 (222)
T3	0.004-0.01 (66-165)	0.004 (66)

DETD Depending upon the analyte of interest and the desired **assay** configuration, the preferred nonspecific binding blocker, as well as the optimization of its concentration and whether it is included as a

component of another **assay** reagent, is selected by empirical techniques which can be performed without undue experimentation by one of ordinary skill in the art of binding **assays**. In only one known instance, i.e., the use of 0.005% dextran sulfate in the **capture** reagent of a competitive digoxin **assay**, was there an inhibition of the binding between the **capture** reagent and solid phase due to the addition of the nonspecific binding blocker.

DETD Ion-**Capture Assay** Devices

DETD As described above, ion-**capture assay** devices may include impermeable solid phase materials such as glass slides, magnetic particles, test tubes and plastic wells. However, it has also been discovered that the entire ion-**capture assay** can be performed in a porous solid phase material. The ion-**capture assay** devices of the present invention specifically involve any suitably absorbent, adsorbent, imbibing, bibulous, non-bibulous, isotropic or capillary possessing material (i.e.,. . .

DETD Possible **assay** devices include, but are not limited to, a conventional chromatographic column, an elongated strip of porous material wherein the fluid. . .

DETD . . . application site. In yet other alternative devices and methods, the indicator reagent can be added to the device as a **separate** reagent solution, either sequentially or simultaneously with the test sample and/or **capture** reagent.

DETD . . . to allow the analyte to migrate from one material to another. The different materials may contain different diffusive or immobilized **assay** reagents, with the individual material being assembled into an elongated strip or flow through pad device. In yet a further. .

. two or more zones of the device may overlap. For example, the sample application zone may also contain a diffusive **assay** reagent (e.g., indicator reagent, **capture** reagent, etc.) which reacts with the analyte to form a complex or reactive product which continues to migrate to other zones in or on the device. In a further example, the sample application zone may contain an immobilized **assay** reagent (e.g., polymer oppositely charged with respect to the **capture** reagent) which immobilizes the **capture** reagent or **capture** reagent complexes for detection. Again, those skilled-in-the-art will readily appreciate the applicability of the present invention to a variety of device formats wherein the indicator reagent is immobilized by directly or indirectly binding to a **capture** reagent conjugate that is in turn immobilized by an oppositely charged solid phase material.

DETD . . . material. Fluid flow contact can include physical contact of the application pad to the porous material as well as the **separation** of the pad from the porous material by an intervening space or additional material which still allows fluid flow between. . .

DETD . . . polyethylene pads and glass fiber filter paper. The material must also be chosen for its compatibility with the analyte and **assay** reagents, for example, glass fiber filter paper was found to be the preferred application pad material for use in a human chorionic gonadotropin (hCG) **assay** device.

DETD In addition, the application pad may contain one or more **assay** reagents either diffusively or non-diffusively attached thereto. Reagents which can be contained in the application pad include, but are not. . . specific binding members, test sample pretreatment reagents and signal producing system components. For example, in a preferred embodiment of an ion-**capture** device an indicator reagent is predeposited in the application pad during manufacture; this eliminates the need to combine test sample and indicator reagent prior to using the device. The **isolation** of **assay** reagents in the application pad also keeps interactive reagents **separate** and

facilitates the manufacturing process. For example, the indicator reagent may be retained in the application pad in a dry. . . at a detection zone, and that indicator reagent which does not become immobilized at the detection zone due to the **assay** reaction will pass from the detection zone.

DETD In a preferred ion-**capture** device, the application pad receives the test sample, and the wetting of the application pad by the test sample will. . . may serve a third function as both an initial mixing site and a reaction site for the test sample and **assay** reagent.

DETD In another preferred embodiment, the application pad contains both the indicator reagent and the **capture** reagent in a dried form. The addition of the test sample reconstitutes the **assay** reagents, thereby enabling their reaction with the analyte and the formation of a charged indicator reagent/analyte/**capture** reagent complex. The complex then migrates from the application pad to the porous test strip material for subsequent reaction with a polymeric material immobilized in a detection zone, wherein that polymeric material is oppositely charged with respect to the **capture** reagent. Alternatively, either the indicator reagent or the **capture** reagent may be contained in the porous test strip material between the application pad and the detection zone. Preferably, the **capture** reagent complex is allowed to form prior to or concurrent with the migration of the **capture** reagent into the detection zone.

DETD . . . pad. The addition of test sample to the overcoated application pad causes the gelatin to dissolve, thereby rehydrating the predeposited **assay** reagent. In an alternative embodiment of the present invention, the reagent containing application pad is dried or lyophilized to increase. . .

DETD In another preferred embodiment, the **assay** devices of the present invention can be further modified by the addition of a filtration means. The filtration means can be a **separate** material placed above the application pad or between the application pad and the porous material. Alternatively, the application pad material.

.

.

DETD . . . reaction of the test sample and the reagent(s) in the application pad. Alternatively, such a layer can contain an additional **assay** reagent(s) which is preferably **isolated** from the application pad reagents until the test sample is added. The flow control layer may also serve to prevent unreacted **assay** reagents from passing to the porous material.

DETD The porous material used in the novel ion-**capture** devices of the present invention may be any suitably absorbant, porous or capillary possessing material through which a solution containing. . . of the optional application pad should be chosen for its ability to premix the test sample and one or more **assay** reagents: fluid flow through a nitrocellulose membrane is laminar and does not provide the more turbulent flow characteristics which allow. . . fiber filter paper are appropriately used as application pads to enable the mixing and reaction of the test sample and **assay** reagents within the application pad. An especially preferred porous material is glass fiber filter paper.

DETD . . . the porous strip material will be a matter of convenience, depending upon the size of the test sample involved, the **assay** protocol, the means for detecting and measuring the signal, and the like. For example, the dimensions may be chosen to. . .

DETD As discussed above, in a binding **assay** the detection site is typically formed by directly or indirectly attaching a charged polymer to the porous material at a. . . reagent to the microparticles encompasses both covalent and non-covalent means, that is adhered, absorbed or adsorbed. It is preferred that ion-**capture** reagents be attached to the microparticles by covalent means.

DETD . . . of the porous material will not adversely affect the performance of the device. As a result, one particularly preferred binding **assay** device uses latex particles, having **capture** reagent attached thereto, distributed in a glass fiber porous material. The distribution of the microparticles or other reagents onto or. . .

DETD The ion-**capture** reagent, signal producing component or reagent-coated microparticles can be deposited singly or in various combinations on or in the porous. . .

DETD Alternatively, the reagent can be distributed over the entire porous material in a substantially uniform manner to form a **capture** site or detection site that substantially includes the entire porous material. In this instance, the extent of signal production along. . .

DETD . . . of the porous material, wherein the reagent within each stripe is directed to a different analyte, thereby forming a multi-analyte **assay** device. As an addition to those devices in which the length or distance of analyte travel is measured, a scale. . .

DETD . . . can be distributed more lightly at one end of the porous material than at the other. In a competitive binding **assay**, this deposition of **capture** reagent in a gradient fashion provides for greater sensitivity at the end of the porous I 5 material having the lighter distribution, because of the more rapid displacement of the indicator reagent from the **capture** reagent binding sites by the analyte.

DETD In alternative embodiments, the appropriate **capture** and signal producing reagents can be distributed in any pattern convenient for detection including, but not limited to, numerals, letters, dots and symbols such as "+/-", "%" or the like which display the detectable signal upon completion of the **assay**. Reaction matrices can optionally be prepared with the **assay** reagents incorporated into the material in an overlapping design, such that the reaction of one reagent completes one portion of. . . portion of the cross. Alternatively, one portion of the design may be visible or detectable prior to performance of the **assay**, with a single reaction completing the overall design. The completion of the vertical portion alone would typically indicate a negative **assay** result, whereas completion of both portions of the detectable design would indicate a positive **assay** result. Any pattern or design may be used, however, wherein the partial formation of the design indicates other than a positive **assay** result and the complete formation of the design indicates a positive **assay** result. Such methods and devices are described in U.S. Pat. No. 4,916,056 the disclosure of which is hereby incorporated by. . .

DETD . . . are spaced from about the proximal end of the porous material to about the distal end, thereby creating a ladder-like **capture** situs configuration. As with the narrow-stripe configuration, the bars and the intervening spaces serve to sharpen the image of the. . .

end

or low end of its concentration range. Another variation of the

parallel

bar configuration involves the use of multiple **capture** or reaction reagents wherein the reagents within the **capture** and detection sites are directed to a different analyte, thereby forming a multi-analyte **assay** device.

DETD . . . solid phase will be a matter of convenience and will depend upon the size of the test sample involved, the **assay** protocol and the means for detecting and measuring the signal. For example, the dimensions may be chosen to regulate the. . .

DETD Predetermined amounts of **assay** reagents can be incorporated within the device, thereby reducing or avoiding the need for additional manipulation by the user. Thus,. . . by being covalently bound to insoluble microparticles which have been deposited in and/or on the

test

strip. More than one **assay** reagent may be present in any given reagent zone or site on the device so long as the reagents do. . .

DETD The various signal display formats or patterns described above can also incorporate **assay** controls to confirm the efficacy of the **assay** reagents, the completion of the **assay** or the proper performance of the **assay**. Such controls are well-known to those skilled-in-the-art. It is also within the scope of this invention to have a reagent, at the distal end of the test strip device, which indicates the completion of the **assay** (i.e., an end of **assay** indicator to signal that the test sample has completed its migration through the device). For example, the completion of the **assay** may be shown by a change of color at the control site upon contact with the test solution, wicking solution. . . or a signal producing component. Reagents which would change color upon contact with an aqueous test solution include the dehydrated **transition metal** salts, such as CuSO.sub.4, Co(NO.sub.3).sub.2, and the like. The pH indicator dyes can also be selected to respond to the. . .

DETD . . . to an application site or by immersing the application site in the test sample. In a sheet-like device having radial **capture** and conjugate recovery sites, the sample is applied to a central application site. Prior to contacting the sample to the solid phase, the sample can also be mixed with additional reagents such as the indicator reagent, **capture** reagent, buffers or wicking reagents (i.e., reagents which facilitate the transport of the test sample through the solid phase). In a further embodiment, the test sample can be applied to one portion of the test strip, upstream of the **capture** site, with one or more of the additional reagents being applied to yet another portion of the test strip upstream. . .

DETD In yet another embodiment, the device can include an additional absorbent material positioned downstream from or beneath the **capture** site. It will be appreciated that the absorbent material can serve to increase the amount of test sample and indicator reagent which passes through the **capture** and detection sites on the solid phase.

DETD . . . it may be necessary to employ a wicking solution, preferably a buffered wicking solution, to facilitate the migration of the **assay** reagent(s) and test sample through the device. When an aqueous test sample is used, a wicking solution generally is not necessary but may be used to improve flow characteristics or adjust the pH of the test sample. In **immunoassays**, the wicking solution typically has a pH range from about 5.5 to about 10.5, and more preferably from about 6.5. . . test sample can be combined prior to contacting the test device, or they can be contacted to the application pad **separately**.

DETD c. Flow-Through **Assay** Devices

DETD . . . analyte of interest. The layer is positioned such that when the device is used in the performance of a binding **assay**, at least a portion of the test sample that contacts the first surface passes through the first surface to an. . .

DETD The flow-through devices may also include an **assay** reagent layer or layers disposed in relation to the first layer, such that when the device is in use, sample fluid passes through the **assay** reagent layer prior to contacting the first surface. The **assay** reagent is typically resolubilized by the addition of test sample to the reagent layer and the reagent is then available for further reaction with the analyte or other reagents housed within the **assay** device. Other embodiments may include a filter layer or a combination filter/reagent layer. Still other devices may involve a removable. . .

DETD The novel flow-through **assay** devices of the present invention,

involve a contact surface wherein a charged polymer is disposed for the nonspecific binding and immobilization of the oppositely charged **capture** reagent and complexes thereof. The device may consist of a layer or a first layer in combination with one or. . . other device

layers described above. For example, one or more pre-reaction layers may

contain the indicator reagent and or the **capture** reagent such that the analyte is allowed to contact the **assay** reagents prior to contacting the ion-**capture** surface of the flow-through device.

DETD In either the flow-through or test strip **assay** devices, one or more **assay** reagents, such as the indicator reagent or **capture** reagent, may be applied to the device during the performance of the **assay**. The preferred embodiments of the present invention, however, involve the incorporation of all necessary **assay** reagents into the **assay** device so that only a test sample, and in some instances a wicking solution or eluting solvent, need be applied. . .

DETD The present invention further provides kits for carrying out binding **assays**. For example, a kit according to the present invention can comprise the **assay** device with its incorporated reagents, and can optionally include a wicking solution and/or test sample pretreatment reagent as described above which are not incorporated in or

on the device. Other **assay** components known to those skilled-in-the-art, such as buffers, stabilizers, detergents, non-specific binding inhibitors, bacteria inhibiting agents and the like

can also be present in the **assay** device and wicking solution.

DETD The following Examples illustrate preferred ways of making the novel materials of the present invention and performing **assay** procedures using those materials. The Examples, however, are intended only to be illustrative, and are not to be construed as. . .

DETD Sandwich **Assay** for Carcinoembryonic Antigen (CEA)

DETD a. Preparation of an Anti-CEA Antibody-PGA **Capture** Reagent

DETD . . . sequence of steps describes the chemistry employed for the preparation of an antibody/polyglutamic acid (PGA) conjugate, i.e., an antibody/anionic polymer **capture** reagent.

DETD . . . with the following procedural modifications. The PDP-PGA was not reduced to the free sulfhydryl prior to the thiopropyl sepharose 6B **isolation**. Instead, the PDP-PGA was dissolved in 0.1M Na phosphate and 1 mM EDTA (pH 6.5) and stirred with thiopropyl sepharose. . .

DETD To trace the number of anionic polymer molecules attached to each **capture** reagent antibody, the TNB-protected PGA was then labeled with an ethylenediamine derivative of fluorescein. The TNB-PGA was loaded with an. . .

DETD . . . thiopropyl-fluorescein-labeled PGA was then reacted with the maleimide derived antibody to yield the antibody/PGA conjugate appropriate for a carcinoembryonic antigen ion-**capture** **immunoassay**. The maleimide-activated antibody (1.0 mg; 6.25.times.10.sup.-9 mole) in 0.1M sodium phosphate (1.0 to 2.0 ml; pH 7.0) was pH adjusted. . .

DETD The largest peak was **assayed** for protein content using Bio-Rad's Bradford **assay** with a bovine IgG standard. The peak contained 95.5 .mu.g/ml protein equating to 5.97.times.10.sup.-7 molar protein (IgG MW 160,000). By. . . this equated to 4.4 PGA molecules conjugated to each antibody. The peak fraction was frozen and subsequently used in the **assay**.

DETD As an alternative to the above **capture** reagent example, a cationic derived antibody could also be formed for use in conjunction with an anionic solid phase material.

DETD . . . conjugate of alkaline phosphatase and anti-CEA antibody fragment, which binds to a different epitope than the antibody specified

in the **capture** reagent. The alkaline phosphatase-labeled anti-CEA antibody fragment was in a buffer containing: 50 mM Tris, 50 mM NaCl, 1.0 mM. . .

DETD d. **Immunoassay** Protocol--Determination of CEA

DETD The indicator reagent (70 .mu.l) was placed into a reaction well. Then, buffered **capture** reagent (20 .mu.l of anti-CEA/PGA conjugate in a buffer of 50 mM Na.sub.2 SO.sub.4, 20 mM sodium phosphate, and 300. . . . and the homogeneous immunoreaction mixture was incubated for 20 minutes at 34.5.degree. C. Four different specimens were run in the **assay**, each of which was a CEA calibrator from the Abbott Laboratories CEA enzyme **immunoassay** kit. An aliquot of each reaction mixture (100 .mu.l) was then applied to the solid phase material, followed by three. . . C. for reaction with the indicator reagent, and the resulting rate of fluorescence was measured. The dose-response results of the **assay** are shown in Table 1. The results demonstrate that as the CEA test sample concentration increased there was a corresponding increase in the formation of **capture** reagent/analyte/indicator reagent complex, and therefore, the amount of detectable label associated with the solid phase increased.

DETD TABLE 1

CEA Ion-**capture** Sandwich **Assay Capture** reagent: anti-CEA antibody-PGA conjugate Indicator reagent: alkaline phosphatase-labeled anti-CEA antibody fragment	
CEA (ng/ml)	Rate (counts/sec/sec)
0	37
4	170
30	931
80	2398

DETD Competitive Inhibition **Assay** of Mouse Immunoglobulin

DETD a. Preparation of an IgG-PGA **Capture** Reagent

DETD c. Binding of the Indicator Reagent to the **Capture** Reagent

DETD . . . diluted in Tris-buffered saline containing 1% fish gelatin [25 mM Tris (hydroxymethyl) aminomethane and 100 mM NaCl, pH 7.5]. The **capture** reagent of PGA/mouse monoclonal antibody conjugate (Pool I of Table 2) was similarly treated. Two hundred microliters of each reagent. . .

DETD TABLE 3

Dose response of **capture** reagent/indicator reagent binding	
PGA/antibody* (.mu.g/ml)	Rate of fluorescence (counts/sec/sec)
10	1559
1	816
0.1	179
0.01	70
0	36

*The initial concentrations of PGAcoupled-antibody before mixing. . .

DETD TABLE 4

Dose response of indicator reagent/**capture** reagent* binding	
Indicator reagent titer**	Rate of fluorescence (counts/sec/sec)
10.sup.2	5062
10.sup.3	796
10.sup.4	93
10.sup.5	10
10.sup.6	5

*The initial concentration of PGAcoupled-antibody before mixing. . .

DETD d. Competitive Inhibition **Assay** for Mouse IgG

DETD The **capture** reagent and indicator reagent were prepared as described above. All of the reagents were diluted in Tris-buffered saline containing 1% fish gelatin. The indicator reagent was diluted 1000-fold from the stock solution, and the **capture** reagent was diluted to ten .mu.g/ml. In a series of test tubes, 150 .mu.l each of appropriately diluted indicator reagent, **capture** reagent, and mouse monoclonal antibody were mixed. The mixtures were incubated at 37.degree. C. for 30 minutes. Aliquots of the. . . at 32.7.degree. C., and the resulting rate of fluorescence was measured. The results of this example illustrating a competitive inhibition **assay** for mouse IgG are shown in Table 5. The results demonstrate that as the mouse monoclonal antibody concentration increased there was a corresponding decrease in the formation of **capture** reagent/indicator reagent complex, and therefore, the amount of detectable label associated with the solid phase decreased.

DETD TABLE 5

Inhibition of indicator reagent binding due to mouse monoclonal antibody **Capture** reagent: PGA/mouse monoclonal IgG conjugate Indicator reagent: alkaline phosphatase-sheep anti-mouse immunoglobulin conjugate	
Mouse IgG (.mu.g/ml)	Rate of fluorescence (counts/sec/sec)
0	110
3.3 .times. 10.sup.-3	106

3.3. . .

DETD Sandwich **Assay** for Human Chorionic Gonadotropin (hCG)

DETD a. Preparation of the **Capture** Reagent

DETD A highly negatively charged albumin derivative was prepared and coupled to anti-hCG antibodies to form the **capture** reagent according to the following procedures.

DETD . . . consisted of an alkaline phosphatase-goat anti-hCG antibody conjugate (prepared by coupling anti-hCG antibody to periodate activated alkaline phosphatase) in an **assay** buffer containing 25 mM Tris (hydroxymethyl) aminomethane, 100 mM NaCl, 1 mM MgCl.sub.2, 0.1 mM ZnCl.sub.2, 0.07% NaN.sub.3, and 1%. . .

DETD c. Sandwich **Immunoassay** Protocol for hCG

DETD The ion-**capture immunoassay** protocol included the use of a solid phase prepared substantially in accordance with the method described in Example 2, the indicator reagent (alkaline phosphatase-goat anti-hCG antibody conjugate), one of two different **capture** reagents (goat anti-hCG Fab'-Sp-SUC.sub.65 -RSA and goat anti-hCG IgG-Sp-SUC.sub.65 -RSA) as prepared in Example 3.a. above, and a purified hCG standard solution. All reagents were appropriately diluted (as determined by a titer curve) in the **assay** buffer. Equal volumes (750 .mu.l) of the indicator reagent and hCG sample

```
     FILE 'EMBASE, SCISEARCH, BIOSIS, MEDLINE, USPATFULL' ENTERED AT 14:28:25
     ON 28 FEB 2001
L1        54031 S (SEPARAT? OR ISOLAT?) AND (CAPTUR? OR AGGLUTINAT? OR ELISA
OR
L2        42332 S (?ACRYLAMIDE? OR ?METHACRYLAMIDE? OR CROSS-LINK?) (5A)
POLYME
L3         5694 S CARBOXYL? (5A) (NITROGEN OR (ITACONIC ACID?) OR (MALEIC
ANHYD
L4          754 S L2 AND L3
L5           37 S L1 AND L4
L6           37 DUP REM L5 (0 DUPLICATES REMOVED)
L7          291 S (TRANSITION METAL?) AND L1
L8            1 S L4 AND L7
L9           55 S L7 AND L2
L10          55 DUP REM L9 (0 DUPLICATES REMOVED)
L11         901 S L1 AND L2
L12          37 S L11 AND L4
L13          37 S L11 AND L3
L14           1 S L13 AND L7
L15          37 S L1 AND L2 AND L3
L16           1 S L15 AND (TRANSITION? METAL?)
L17          76 S (?ACRYLAMIDE?) AND (TRANSITION METAL?) AND (CAPTUR?) AND
(?AS
L18          57 S L17 AND (ISOLAT?)
L19          57 DUP REM L18 (0 DUPLICATES REMOVED)
L20        3851 S (TARGET? (3A) ISOLAT?)
L21           0 S L4 AND L20
L22          87 S L2 AND L20
L23           6 S L22 AND L7
L24           6 DUP REM L23 (0 DUPLICATES REMOVED)
L25          87 S L20 AND L2
L26           0 S L25 AND L3
L27           0 S L25 AND L3
L28          12 S L25 AND (TRANSITION METAL?)
L29          12 DUP REM L28 (0 DUPLICATES REMOVED)
```

L10 ANSWER 42 OF 55 USPATFULL
ACCESSION NUMBER: 95:78110 USPATFULL
TITLE: Magnetically assisted binding assays using magnetically
labeled binding members
INVENTOR(S): Rohr, Thomas E., Gurnee, IL, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5445971	19950829
APPLICATION INFO.:	US 1994-348780	19941201 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-161376, filed on 2 Dec
1993, now abandoned which is a continuation-in-part of Ser. No. US 1992-854151, filed on 20 Mar 1992, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Scheiner, Toni R.
ASSISTANT EXAMINER: Wolski, Susan C.
LEGAL REPRESENTATIVE: Bach, Mark C.
NUMBER OF CLAIMS: 2
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 25 Drawing Figure(s); 12 Drawing Page(s)
LINE COUNT: 1755
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides devices for performing binding assays. Such devices comprise (i) a reaction vessel where unbound and immobilized magnetically-labeled reagent are produced in relation to the amount of said analyte in said test sample; (ii) a **separation** means for partitioning said immobilized magnetically-labeled reagent and said bound magnetically-labeled reagent; (iii) a magnetic field generator means for the application of a magnetic field to said magnetically-labeled reagent; and (iv) a measurement means to assess the effect of said magnetic field on said magnetically-labeled reagent as a measure of the presence or amount of said analyte in said test sample. The device provided by the instant invention can run, for example, direct indirect, competitive, inhibition and sandwich assay formats.

AB . . . and immobilized magnetically-labeled reagent are produced in relation to the amount of said analyte in said test sample; (ii) a **separation** means for partitioning said immobilized magnetically-labeled reagent and said bound magnetically-labeled reagent; (iii) a magnetic field generator means for the. . .

SUMM Diagnostic **assays** have become an indispensable means for detecting analytes in test samples by using the mutual reaction between the analyte and. . . tags or labels attached to antibodies, which in turn bind to the analyte of interest, are employed in such diagnostic **assays**, wherein the detection of the resultant labeled antibody/analyte complex, or of the labeled antibody which remains unbound, is used to. . .

SUMM Two commonly used binding **assay** techniques are the **radioimmunoassay** (RIA) and the enzyme **immunoassay** (EIA), both of which employ a labeled binding member. The RIA uses a radioactive isotope as the detectable tag or. . .

SUMM . . . may be difficult to purify and conjugate to binding members,

and may be unstable during storage at room temperature. Enzyme **immunoassays** are also unsatisfactory in that the methods typically require complex incubations, multiple liquid additions and multiple wash steps. Moreover, even. . .

SUMM More recently, **assay** techniques using metallic sol particles as visual labels have been developed. In these techniques, a metal (e.g., gold, silver, platinum),. . . Generally, the binding member to be labeled is coated onto the metal sol particles by adsorption, and the particles are **captured** or aggregated in the presence of analyte. Although the metal sol particles have the advantage of producing a signal that. . . are difficult to quantitatively measure.

The metallic particles also have a limited color intensity, and therefore limited sensitivity in some **assays**. In addition, the surfaces of inorganic metallic colloid particles, such as gold, do not readily accept the covalent attachment of binding members. Thus, during use in a binding **assay**, care must be taken so that the adsorbed binding members are not removed from the inorganic particles through the combination. . .

SUMM The present invention advantageously uses a magnetically-attractable material as a detectable label in binding **assays**. The magnetic label is subjected to a magnetic field and the label, in turn, displays a resultant force or movement. . .

SUMM . . . is selected to bind (i) the analyte or (ii) the first binding member, to thereby provide for competitive and sandwich **immunoassay** formats.

SUMM . . . solid-phase reagent in relation to the amount of analyte present in the test sample. The unbound magnetically-labeled reagent can be **separated** from the magnetically-labeled reagent bound to the solid-phase reagent prior to or during the application of a magnetic field to. . .

SUMM . . . unbound and immobilized magnetically-labeled reagent are produced in relation to the amount of analyte in the test sample; (ii) a **separation** means for partitioning the immobilized magnetically-labeled reagent and the unbound magnetically-labeled reagent; (iii) a magnetic field generator means for the. . . the test sample. It will be understood, of course, that the magnetic field generator means can also serve as the **separation** means and that suitable magnetic field generator means comprise permanent magnets and electromagnets.

DRWD FIG. 6 illustrates the results of a binding **assay** using a magnetically-labeled reagent as the detectable label.

DRWD FIG. 7 illustrates the results of a binding **assay** using a magnetically-labeled reagent as the detectable label, plotted as an inhibition curve.

DRWD . . . the effect of the repeated approach and withdrawal of a magnetic field from a solid phase containing antibody-coated magnetic particles **captured** by an immobilized antibody.

DRWD . . . a single cycle of the approach and withdrawal of a magnetic field from a solid phase containing antibody-coated magnetic particles **captured** by an immobilized antibody.

DRWD FIG. 11 illustrates an inhibition curve from a magnetically assisted **immunoassay**.

DETD . . . analyte complexes or assay reagents become bound and from which unreacted assay reagents, test sample or test solutions can be **separated**. The solid phase generally has a binding member immobilized on or in its surface to form a "solid-phase reagent", that. . .

DETD . . . of the solid phase before, during or after contacting the first

solid phase component with the test sample and/or other **assay** reagents. In most embodiments, however, the binding member is bound or attached to a single solid phase component prior to contacting the thusly formed solid-phase reagent with the test sample or other **assay** reagents.

DETD . . . term "ancillary binding member", as used herein, refers to any member of a binding pair which is used in the **assay** in addition to the binding members of the magnetically-labeled reagent or solid-phase reagent. For example, in instances where the analyte. . . of interest. As it will be understood, of course, one or more ancillary binding members can be used in an **assay** and such ancillary binding member(s) can be attached to the magnetically-labeled reagent or solid-phase reagent either before, during or after the magnetically-labeled reagent or solid-phase reagent is contacted with a test sample or other **assay** reagent. The ancillary binding member can be incorporated into the **assay** device or it can be added to the device as a **separate** reagent solution.

DETD . . . invention results from the unexpected andsurprising discovery that, when a magnetically-responsive material is used as a label in a binding **assay**, it is possible to detect the presence or amount of either or both of the free or bound label by. . .

DETD Conventional heterogeneous binding **assay** formats require vigorous washing of the solid phase to **separate** bound and unbound labeled reagent and to suppress the nonspecific binding of materials to the solid phase. Such wash steps complicate the **assay** protocol and restrict the **assay** to the useof binding pair members having high affinity, i.e., a binding strength thatwill withstand such physical manipulation. Conversely, the present invention avoids the need for complex washing steps in binding **assays** because unbound or non-specifically bound label can be **separated** from the reaction mixture by the application of a first magnetic field prior to thedetection of specifically bound label by. . . of control that is possible over the magnetic field permits the use of a first field that is suitable to **separate** free or non-specifically bound label from a reaction mixture without affecting specifically bound label. In turn, this permits the use. . .

DETD In conventional particle **agglutination assays**, binding members of low affinity can be used because several binding sites on each member can cooperate to give high avidities, and the absence of wash steps allows weak associations to be maintained while simplifying the **assay** format. Signal amplification results from the fact that the interaction of a few binding sites can cause the aggregation of. . . size and mass than the original members, and thereby provide a macroscopic change which can be interpreted visually. However, particle **agglutination assays** are often difficult to interpret, do not yield quantitative results, and are not readily amenable to automation.

DETD The present invention solves the aforementioned problems of conventional heterogeneous and **agglutination assays** by placing the magnetic label in a magnetic field, and measuring the consequences of the magnetic force exerted upon the label to provide a qualitative or quantitative **assay** readout. The force affect of the magnetic field upon the magnetic label enhances the detection of the **captured** or aggregated magnetic label while suppressing non-specific interference from any non-magnetic substances. For example, the nonspecific binding of extraneous substances. . . the detection of the weight of bound analyte as is determined by conventional gravimetric analyses), the present invention provides binding **assay** signal enhancements of about nineorders of magnitude and is sufficient to detect analyte concentrations in the femtomolar (10.sup.-15 mole or. . . limited to those used to sense force changes

in an atomic force microscope; and the like. This enables very sensitive
assays and obviates the need for amplification of the label as required in many conventional **assays**.

DETD . . . means of an electromagnetic or a movable permanent magnet. A field intensity can be chosen which is optimalfor a particular **assay** and particular binding reagents, such that a field sufficient to remove unbound and non-specifically bound magnetic label canbe applied without. . . the binding members. This provides the opportunity to use binding members having lowerbinding affinities than those typically found in binding **assays**.

DETD It is to be understood that the aforementioned advantages permit the **assaysto** be readily adapted to computer control. In addition, the intensity of the magnetic field can be precisely manipulated to disrupt. . .

DETD (a) **Assay** Reagents

DETD . . . can bind, carry or be modifiable so as to attach to a binding member which will in turn bind another **assay** reagent or a component present in a test sample. It is also preferred that the label be magnetically attractable to. . . understood, of course, that the choice involves consideration of the analyte to be detected and the desired optimization of the **assay** technique.

DETD . . . of a single
material core
particle of magnetically-
with a responsive material with a
nonmagnetic
coating of a water-insoluble
coating **cross-linked polymeric**
material
having reactive groups at the
surface thereof
an organic polymer particle with
a ferrite coating
a sphere of thermoplastic
. . . form of iron and
a water-soluble polymer having
available coordination sites
(free electron pair for a
coordinate bond with a
transition metal atom)
an organic, inorganic or
synthetic polymer matrix
containing a magnetically-
attractable material
magnetizable particles of a size
less than. . . form of iron and
a water-soluble polymer having
available coordination sites
(free electron pair for a
coordinate bond with a
transition metal atom)
an organic, inorganic or synthetic
polymer matrix containing a
magnetically-attractable material
a continuous phase of a water-
insoluble polymeric. . .

DETD . . . test strip for chromatographic or thin layer chromatographic techniques in which one or all of the reagents are contained in **separate** zones of a single strip of solid phase material; or any absorbent material well knownto those skilled in the art.

DETD According to such assay protocols, after a period suitable for binding, theunbound magnetically-labeled reagent can be **separated** from the bound magnetically-labeled reagent. It will be understood, of course, that the **separation** of bound and unbound magnetically-labeled reagent may involve the complete removal of the

unbound magnetically-labeled reagent from the reaction mixture and/or from that magnetically-labeled reagent which is immobilized to the solid-phase reagent. The **separation** of bound and unbound magnetically-labeled reagent may also involve the segregation of the unbound magnetically-labeled reagent from the immobilized magnetically-labeled. . .

DETD . . . for performing magnetically assisted binding assays as taught herein. Accordingly, such devices preferably comprise (i) a reaction vessel; (ii) a **separation** means for **separating** the immobilized magnetically-labeled reagent from the unbound magnetically-labeled reagent; (iii) a magnetic field generator means for the application of a. . .

DETD **Separating** the bound magnetically-labeled reagent from the unbound magnetically-labeled reagent can be accomplished by any means suitable forpartitioning the unbound and bound magnetically-labeled reagent. For example, a vibratory or tilting means could be used to effectuate the **separation**. Preferably, the magnetically-labeled reagent that is not immobilized to the solid-phase reagent is **separated** from the solid phase by the application of a magnetic field which is sufficient to move unboundmagnetically-labeled reagent, but not. . . of the reaction mixture such that unbound magnetically-labeled reagent is sequestered at the air/liquid interface of the reaction mixture, thereby **separating** unbound magnetically-labeled reagent from the immobilized reagent. In a further embodiment, the unbound reagent can be moved away from the. . .

DETD . . . and electromagnets. It will also be understood, of course, that the magnetic field generator means may also be used to **separate** the unbound or free magnetically-labeled reagent from the bound or immobilizedreagent.

DETD While various devices and **assay** protocols are contemplated by the present invention, the following protocols represent examples, and are not intended to be limited to, two sandwich **assay** formats using the magnetically assisted detection of a magnetically-labeled reagent of the present invention. In this regard, the following protocols,. . .

DETD . . . magnetically-labeled reagent to form a second reaction mixture whereby the magnetically-labeledreagent becomes immobilized upon the solid-phase reagent by binding the **captured** analyte (the proportion of magnetically-labeled reagent that becomes bound to the solid-phase reagent is directly related to the amountof analyte. . .

DETD . . . magnetic field intensity increases, the weaker association of non-specifically bound magnetically-labeled reagent with the well bottom will be overcome, thereby **separating** it from the specifically bound magnetically-labeled reagent.

DETD Magnetically Assisted Avidin-Biotin Binding **Assay**

DETD The following reagents and samples were used in a binding **assay** :

DETD . . . to bind to the available biotin-binding sites on the avidin moieties of the avidin-coated magnetic particles, thereby inhibiting the subsequent **capture** of the magnetically-labeled reagent by the immobilized biotin on the well bottom. The degree of inhibition depended on the concentration. . .

DETD FIG. 6 illustrates the **assay** results. The balance means detected a decreasing force change, from 12 milligrams to zero milligrams, as the free biotin concentration in the test sample was increased from 0 nanograms/milliliter to 125 nanograms/milliliter (80 .mu.l **assayed**). Thus,as the amount of free biotin was increased in a test sample, the amount of magnetically-labeled reagent which bound to. . .

DETD FIG. 7 illustrates the **assay** results plotted as a percent

inhibition of themagnetically enhanced weight of the **captured** magnetically-labeled reagent resulting from the presence of free biotin in the test sample. Fifty percent (50%) inhibition was observed at a free biotin concentration of 40nanograms/milliliter. From these results,
it was determined that the **assay** configuration provided an **assay** for free biotin in the test sample with a sensitivity in the femtomolar range.

DETD To further explore the potential of the magnetically assisted magnetic label **assay** concept, a Cahn Model D-200 electronic microbalance (Cahn Instruments Incorporated, Cerritos, Calif.) was used.
This balance consists of a balance. . .

DETD . . . on the well bottom was inhibited. The quantity of magnetically-labeled reagent remaining bound to the solid phase, after the magnetic **separation** of unbound magnetically-labeled reagent, was measured by placing the well on a balance pan, zeroing the balance, and then moving. . .

DETD . . . IgG (overcoated with BSA), exhibited less than a one milligram weight change, thereby indicating that enough magnetically-labeled reagent had been **captured** by the immobilized antibody on the well bottom to decrease by two-thirds the magnetic response measurement due to the unbound. . .

DETD . . . to the liquid surface under the influence of the magnetic fieldand display a greater magnetic responsiveness. In this way, inhibition **immunoassays** similar to that shown in FIG. 9-11 could be monitored by apparent weight change caused by magnetic particle
levitation, i.e.,. . .

DETD Magnetically Assisted Binding Measurements in a Two Particle **Assay**

DETD An alternative **assay** method can involve the use of a particulate solid phase. The magnetically-labeled reagent comprises a binding member conjugated to a. . .

DETD . . . a much slower rate in the magnetic field, thereby providing for
the discrimination between bound andfree magnetically-labeled reagent. After the **separation** of the unbound magnetically-labeled reagent, that magnetically-labeled reagent that is bound to the non-paramagnetic particles is subjected to analysis using. . .

DETD The magnet-balance configuration described in Example 9 allows the performance of assays where the unbound particles are not **separated** from the reaction vessel containing the bound particles. As illustrated in FIG.17a, a conical magnet 60 is placed on the. . .

DETD . . . contacted the underside of the cover slip thereby leaving no air space. The unbound magnetically-labeled reagent in the well was **separated** from the bound magnetically-labeled reagent by elevating the reaction vessel in the proximity of a magnet 60 affixed to
the. . .

DETD . . . This approach avoids the necessity of removing the unbound particles from the reaction vessel before the read step, allowing the **separation** and read step to be performed simultaneously.

CLM What is claimed is:
. . . and immobilized magnetically-labeled reagent are produced in relation
to the amount of said analyte in said test sample; (b) a **separation** means, operatively positioned with respect to said reaction vessel, for partitioning said immobilized magnetically-labeled reagent and said unbound magnetically-labeled reagent;. . .
2. The assay device according to claim 1 wherein said **separation** means comprises a magnetic field generator means which is the same as or
different from said magnetic field generator means. . .

L10 ANSWER 49 OF 55 USPATFULL

ACCESSION NUMBER: 89:51831 USPATFULL

TITLE: Polymerization-induced **separation** immunoassays

INVENTOR(S): Nowinski, Robert C., Seattle, WA, United States
Hoffman, Allan S., Seattle, WA, United States

PATENT ASSIGNEE(S): Genetic Systems Corporation, Seattle, WA, United States
(U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 4843010	19890627
APPLICATION INFO.:	US 1984-574558	19840127 (6)

DISCLAIMER DATE: 20020416

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1983-550929, filed on 10 Nov 1983, now patented, Pat. No. US 4511478

DOCUMENT TYPE: Utility

PRIMARY EXAMINER: Warden, Robert J.

ASSISTANT EXAMINER: Benson, Robert

LEGAL REPRESENTATIVE: Neeley, Richard L.

NUMBER OF CLAIMS: 20

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 5 Drawing Figure(s); 5 Drawing Page(s)

LINE COUNT: 789

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Immunoassay methods and compositions are disclosed for the detection of analytes in fluid samples. The disclosure provides conjugates of analytes or reactants with polymerizable organic monomers. Specific binding reactions between reactants are detected by means of resporter/reactant conjugates. Free and specifically-bound reporter/reactant conjugates are **separated** by a polymerization reaction which renders the polymerized monomers insolule.

TI Polymerization-induced **separation** immunoassays

AB . . . organic monomers. Specific binding reactions between reactants are detected by means of resporter/reactant conjugates. Free and specifically-bound reporter/reactant conjugates are **separated** by a polymerization reaction which renders the polymerized monomers insolule.

SUMM . . . generally to immunoassay methods and more particularly to an immunoassay in which a polymerization reaction is used to effect a **separation** of the specific reactants.

SUMM **Immunoassays** have found widespread application in the field of clinical diagnostics for the detection and measurement of drugs, vitamins, hormones, proteins,. . .

SUMM **Immunoassays** generally incorporate antibodies and antigens as reactants, at least one of which is labeled with a signal producing compound (e.g.. . .

SUMM **Immunoassays** can be divided into two general categories, homogeneous and heterogeneous. In a homogeneous **immunoassay**, the signal emitted by the specifically-bound labeled reactant is different from the signal emitted by the free labeled reactant. Hence, bound and free can be distinguished without physical **separation** .

SUMM The archetypal homogeneous **immunoassay** is the enzyme-multiplied **immunoassay** technique (EMIT), which is disclosed in U.S. Pat. No. 3,817,837. In this technology, analyte present in patient sample and analyte/enzyme. . . its enzymatic activity. Hence, the amount of enzyme activity is proportional to the amount of analyte in the sample. Homogeneous **immunoassays** have the advantage of being rapid, easy to perform, and readily amenable to automation. Their principal disadvantages are that they. . .

SUMM In a heterogeneous **immunoassay**, the signal emitted by the bound labeled reactant is indistinguishable from the signal emitted by the free labeled reactant. Therefore, a **separation** step is required to distinguish between the two.

SUMM Typical heterogeneous **immunoassays** include the **radioimmunoassay** (RIA) and the enzyme-linked **immunosorbent assay** (**ELISA**). In the RIA, radiolabeled analyte and analyte present in patient sample compete for a limited amount of immobilized (solid phase). . . . remove unbound labeled analyte and either the bound or the free fraction is analyzed for the presence of labeled reactant. **ELISA assays** are performed analogously. In this case, though, the signal is an enzyme instead of a radioisotope. Heterogeneous **immunoassays** typically employ at least one reagent immobilized on a solid phase. Since the kinetics of reaction between an immobilized antibody. . . incubation times are frequently required. When the multiple wash steps often needed are considered, it can be appreciated that heterogeneous **assays** tend to be time-consuming and labor-intensive. However, they are in general more sensitive than homogeneous **assays** and less prone to interferences, since interfering substances can be removed in the wash steps.

SUMM Solids used to immobilize reactants in **immunoassays** have included controlled pore glass and preformed **polymers** such as polyvinyls, **polyacrylamides**, polydextrans and polystyrene.

SUMM Numerous **separation** methods are known in the art and have been used in heterogeneous **immunoassays**. These include centrifugation, filtration, affinity chromatography, gel permeation chromatography, etc.

SUMM The homogeneous **immunoassay** methods of the prior art are generally prone to interferences, of limited sensitivity and have a limited range of antigen sizes. The heterogeneous **immunoassays** of the prior art, while increasing the sensitivity and minimizing interferences, tend to be time consuming and labor intensive. These difficulties generally arise from the added step of physical **separation** and the need for numerous washes to decrease background interference.

SUMM There is a need in the art for an **immunoassay** method which is sensitive to sub-micromolar concentrations of analyte; which has fast reaction kinetics; and which minimizes the number of. . .

SUMM . . . structures (polymers). Synthetic polymers can be formed from a single monomeric species (homopolymer) or from a mixture of different monomers (co-**polymer**). Linear, branched, or **cross-linked** structures are possible. By varying the chemical composition or ratios of the monomers, it is possible to form either soluble. . .

SUMM . . . a monomer/reactant conjugate in order to form a monomer/reactant conjugate-analyte complex and providing a reporter for said monomer/reactant conjugate--analyte complex, **separating** said reporter-labeled complex by initiating polymerization of the monomer/reactant conjugate--analyte complex and detecting the incorporation of reporter into said polymerized. . .

SUMM . . . specific binding complement (i.e., its appropriate antigen or antibody counterpart bound through specific antibody/antigen interactions) can be rapidly and conveniently **separated** from solution by initiating a polymerization reaction. In contrast to the monomer/reactant conjugate and its specifically bound complement, other components of the immunoassay remain in free solution. Thus, this method provides an effective single-step **separation** of specifically bound and free reactants.

SUMM The polymerization-induced **separation** immunoassays of the instant invention are believed to offer several advantages over prior art homogeneous and heterogeneous immunoassay methods. Because of the **separation** achieved by polymerization of the monomer/reactant conjugate, the immunoassay of this invention can achieve the sensitivity typical of state-of-the-art heterogeneous. . .

DETD . . . the immunoassay of analytes in biological fluids utilize conjugates of reactants with monomers or signal-producing compounds (monomer/reactant or reporter/reactant conjugates). **Separation** of free from specifically bound reporter/reactant is effected by a polymerization reaction.

DETD . . . antigen to the first antibody. Analyte present in sample and monomer/analyte conjugate compete for a limited amount of reporter/reactant. Polymerization-induced **separation** of free from specifically-bound reporter/reactant enables the detection and measurement of analyte initially present in the sample. This configuration is. . .

DETD . . . prior to measuring the amount of reporter associated with the polymer particle. Similarly, if desired, the polymer particles can be **separated** from solution and the reporter associated with them eluted prior to detection or measurement.

DETD . . . but in this case the reactant is an antibody to the monomer/reactant-analyte complex rather than to the analyte. Following polymerization-induced **separation** of free from specifically-bound reporter/reactant, the presence or amount of reporter/reactant specifically bound to the polymer particles is determined. In. . .

DETD . . . be appreciated that a mixture of copolymerizable monomers can be conjugated to the reactant or analyte and which are thereafter **separated** by polymerization.

DETD . . . discussed, alkyl acrylates or methacrylates where the alkyl radical contains from 1 to 8 carbons, acrylonitrile and vinyl acetate. Also, **cross-linking** compounds may be co-**polymerized** with the monomer/reactant conjugate. Such cross-linking compounds may include, for example, N,N'-methylenebisacrylamide or a di-, tri-or tetramethacrylate or acrylate. The. . .

DETD **Separation** of the specifically bound from the free reactants is accomplished by polymerization of the monomer/reactant conjugate. Polymerization or copolymerization with. . .

DETD . . . by employing photoinitiators, such as azodiisobutyro-nitrile, azodiisobutyroamide, benzoin methyl ether, riboflavin, thiazine dyes such as methylene blue and eosin, and **transition metals** such as ferric chloride or diazidotetramminecobalt (III) azide, in combination with ultraviolet and/or visible light irradiation of the reaction system.

DETD . . . and the mixture was stirred for 30 minutes at 4.degree. C. Upon addition of chloroform (50 mL), the mixture was **separated** into layers, and the organic layer was extracted successively with water (generally 5 times with 50 mL each time) until. . .

DETD . . . of the monomer/reactant conjugate was then analyzed by isoelectric focusing. In this procedure, the polypeptide subunits of the proteins were **separated** according to their isoelectric point, or pH at which they had no net positive or negative charge. For this purpose,. . . monomer/reactant conjugate were first dissociated in the presence of 3% (w/v) sodium dodecyl sulfate (SDS) and 5% (v/v) 2-mercaptoethanol and **separated** on the basis of molecular weight by electrophoresis in an SDS-polyacrylamide slab gel. The **separated** heavy and light chains of the reactant were cut out from the gel and analyzed further by isoelectric focusing in. . . of antibodies are glycoproteins which contain intrinsic variations in their sialic acid content, each heavy and light chain can be **separated** by charge into a characteristic family of bands, with each band containing a polypeptide and differing amounts of sialic acid.. . .

DETD To show that the purified monomer/reactant conjugate was still active, it was tested in an enzyme linked **immunosorbent assay** (**ELISA**), and the results indicated no loss of specific binding capacity. For this purpose, human IgG (which contain the same kappa chain antigen as human IgM) was adsorbed to the surfaces of wells in a

micro **ELISA** plate (96 wells). The wells were washed, residual nonspecific adsorbing sites on the plastic surface were blocked with bovine serum. . . for horseradish peroxidase, o-phenylenediamine and hydrogen peroxide. Dilute aqueous sulfuric acid was added to stop the reaction, the plates were **assayed** on a micro **ELISA** reader, and the optical densities of each dilution of monomer/antibody conjugate compared with that of the control antibody. On a. . .

DETD The final step in the assembly of the components of a simultaneous sandwich **immunoassay** system was the identification of a second antibody (2C3, which reacts with the mu heavy chain of human IgM) that. . . a column of Sephadex.RTM. G-25 in phosphate buffered saline to which 0.5M NaCl and 0.1% NaN.sub.3 had been added. This **separated** the fluorescein labeled antibody from any free FITC that remained in solution. The peak was collected in a volume of. . .

DETD . . . commonly used in immunoassays to solubilize biological substances, this indicates that it will be possible to utilize detergents in polymerization-induced **separation** immunoassays without interference.

CLM What is claimed is:

. . . complex or to said anaylte, for labeling said monomer/reactant conjugate--analyte complex, wherein said monomer is an addition polymerizable monomer; (b) **separating** said labeled complex by initiating polymerization of the monomer/reactant conjugate--complex; (c) detecting the incorporation of reporter into said polymerized complex. . .

. . . mixture with a reporter/reactant conjugate capable of specifically binding analyte to form reporter-labeled analyte complex and reporter-labeled monomer/analyte complex; (c) **separating** said reporter-labeled monomer/analyte complex by initiating polymerization of

the monomer/analyte-conjugate complex; and (d) detecting the incorporation of reporter into said. . .

L10 ANSWER 50 OF 55 USPATFULL

ACCESSION NUMBER:	89:1178 USPATFULL
TITLE:	Magnetic-polymer particles
INVENTOR(S):	Owen, Charles S., Swarthmore, PA, United States Silvia, John C., Warminster, PA, United States D'Angelo, Louis, Berlin, NJ, United States Liberti, Paul A., Churchville, PA, United States
PATENT ASSIGNEE(S):	Immunicon Corporation, Huntingdon Valley, PA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 4795698	19890103
APPLICATION INFO.:	US 1986-906521	19860916 (6)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1985-784863, filed on 4 Oct 1985, now abandoned	
DOCUMENT TYPE:	Utility	
PRIMARY EXAMINER:	Nucker, Christine M.	
LEGAL REPRESENTATIVE:	Ratner & Prestia	
NUMBER OF CLAIMS:	44	
EXEMPLARY CLAIM:	32	
LINE COUNT:	842	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A magnetic-polymer particle, useful in immunoassay techniques and various other biological/medical applications is produced by coprecipitation of **transition metals** in the presence of a polymer having available coordination sites. These particles are capable of forming stable aqueous suspensions and may be easily resuspended after agglomeration.

AB A magnetic-polymer particle, useful in immunoassay techniques and various other biological/medical applications is produced by coprecipitation of **transition metals** in the presence of a polymer having available coordination sites. These particles are

capable of forming stable aqueous suspensions and. . .

SUMM . . . active magnetic particles may find use in a variety of preparative and diagnostic techniques. Among these is high gradient magnetic **separation** (HGMS) which uses a magnetic field to **separate** magnetic particles from suspension. In instances where these particles are attached to biological materials of interest (e.g. cells, drugs), the material of interest may thereby be **separated** from other materials not bound to magnetic particles.

SUMM . . . An atom in a molecular structure which has a "free" electron pair capable of forming a coordinate bond with a **transition metal** atom.

SUMM . . . the preparation of suspendable and resuspendable magnetic-polymer particles and the particles produced thereby. Such particles exhibit useful properties, particularly in **immunoassays** wherein the particles are prepared with a particular biofunctional ligand and are subsequently **separated** by high gradient magnetic **separation** techniques.

SUMM . . . various biofunctional groups may be incorporated into the particles in order to yield an effective biofunctional reagent for use in **immunoassay**, cell **capture**, enzyme immobilization reactors, NMR imaging, and other diagnostic and analytical techniques.

SUMM . . . other than iron in the coprecipitation reaction. In particular, Fe(III) may be replaced by any of a wide range of **transition metal** ions. In some cases, iron may be completely supplanted by appropriately selected **transition metal** iron. In many cases, the use of metals other than ion produces colored particles ranging from white to dark brown.

SUMM Subsequent to precipitation and resuspension of the magnetic-polymer particles, they may be treated with a bifunctional reagent in order to **cross-link** reactive sites present on the **polymer**. This **cross-linking** may be effective as either an intra-particulate cross-linking in which reactive sites are bound on the same particle, or may be a reaction of an extra-particulate ligand which is then **cross-linked** to the **polymer** on a given particle. In the second case, a bifunctional reagent having a relatively short distance between its two functional. . .

SUMM In selecting the **transition metals** to be employed in the coprecipitation reaction, several criteria appear to be important. First, the final compound must have one. . .

DETD In addition to the list above, Fe(II) may be used in combination with selected **transition metal** ions whose electromotive potential is insufficient to oxidize the Fe(II) to Fe(III). Of the above listed metals, only V(III) is. . .

DETD . . . minutes 3.1 mg dithiothreitol (DTT) was added to convert the SPDP to its free sulfhydryl form. The reacted Ab was **separated** (desalted) on a small gel filtration column.

DETD Demonstration of Magnetic **Immunoassay** Using Particles Coupled to an Antigen

DETD . . . containing enzyme substrate. After 15 minutes incubation, the buffer was eluted and the amount of reaction product generated by enzyme **captured** on the filter bed was determined by measuring the optical density.

DETD The above procedure formed the basis of a competitive **immunoassay** for human IgM. When small amounts of free IgM were added to the incubation mixture, the uptake of enzyme by. . . the incubation mixture was graphed to produce a calibration curve which allowed the process to be used as a competitive **immunoassay** to measure unknown amounts of IgM. The sensitivity was approximately 0.15 mg/ml (the concentration of IgM which resulted in 50% reduction in the specific **capture** of enzyme activity on the filter).

DETD . . . goat-anti-rabbit-Ig activity by hemagglutination. A series of microtiter wells was set up containing sheep red blood cells (SRBC) and a sub-**agglutinating** concentration of rabbit antibody against SRBC. In each series, particles were added in concentrations which decreased two-fold in each successive well in the series. After several hours, the maximum well in which **agglutination** could be seen was read for each series. More active particles **agglutinated** at lower concentrations (larger well numbers). A table of results for the 12 preparations tested is given below.

DETD (v) The particles, when coupled to specific biofunctional ligands, may be used in magnetic **immunoassays** for biological materials of interest.

DETD . . . novel magnetic-polymer particles and methods for making them. These particles are useful in a variety of biological/medical fields including cell **capture**, use as a contrast reagent for NMR imaging, immobilized enzyme reactors, **immunoassay**, and other analytical and diagnostic techniques.

CLM What is claimed is:

. . . preparation of magnetic-polymer particles comprising the steps of: (a) combining a first aqueous solution of at least two species of **transition metal** ions capable of reacting with each other to form a magnetic precipitate and a polymer having available coordination sites in proportions adapted to produce a resuspendable product; (b) reacting said **transition metal** ions in the presence of said polymer to form a magnetic precipitate comprising magnetic-polymer particles; (c) recovering said magnetic-polymer particles.

. . .

12. The process of claim 1 wherein the **transition metal** ions in solution comprise a mixture of Fe(II) and Fe(III).

. . . suspension to increase its ionic strength to a molarity adapted to cause agglomeration and precipitation of said magnetic-polymer particles; (f) **separating** said agglomerated precipitated particles; (g) resuspending said magnetic-polymer particles in an aqueous solution of low ionic strength adapted to allow. . .

. . . 32. A process for the assay of a predetermined species comprising the

steps of: (a) preparing an aqueous solution of **transition metal** ions capable of reacting to form a magnetic precipitate and a polymer having available coordinate sites in proportions adapted to. . .

37. The process of claim 1 wherein said **transition metal** ions are chosen from the group consisting of those which form coprecipitates having at least one unpaired electron, and having.

. .

38. The process of claim 1 wherein said **transition metal** ions comprise a pair of ions selected from the group of: Co(II)+Ga(III) Ga(III)+Er(III) Co(II)+Ru(III) Ga(III)+Ru(III) Co(II)+Mn(II) Ga(III)+Mn(II) Ga(III)+V(III) Co(II)+V(III) Ga(III)+Mo(V).

. .

39. The process of claim 1 wherein said **transition metal** ions comprise Fe(II) and a **transition metal** ion having an electromotive potential insufficient to oxidize Fe(II) to Fe(III).

40. The **assay** of process of claim 32 wherein said suspension is further treated with an agglomerating agent to promote agglomeration of said. . .

41. The **assay** process of claim 40 wherein said agglomerating agent is a salt of a Group II metal.

42. The **assay** process of claim 40 wherein said agglomerated particles are filtered by **capturing** said particles using a hand-held permanent magnet.

43. The **assay** process of claim 40 wherein the amount of agglomeration caused by said agglomerating agent is sufficient to allow **capture** of said agglomerated particles by a hand-held permanent magnet in 3 minutes from 1 ml. of suspension, but insufficient to. .

. . . preparation of magnetic-polymer particles comprising the steps of: (a) combining a first aqueous solution comprising at least two species of **transition metal** ions capable of reacting with each other to form a magnetic precipitate, and a polymer having biofunctional activity and having available coordination sites in proportions adapted to produce a resuspendable precipitate product having biofunctional activity; (b) reacting said **transition metal** ions in the presence of said polymer to form a magnetic precipitate comprising a complex of said metal and said. . .

L19 ANSWER 13 OF 57 USPATFULL
ACCESSION NUMBER: 2000:21439 USPATFULL
TITLE: Methods of **isolating** biological target materials using silica magnetic particles
INVENTOR(S): Smith, Craig E., Oregon, WI, United States
York, Charles K., Madison, WI, United States
PATENT ASSIGNEE(S): Promega Corporation, Madison, WI, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6027945	20000222
APPLICATION INFO.:	US 1997-785097	19970121 (8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Housel, James C.
ASSISTANT EXAMINER: Phan, Quang N.
LEGAL REPRESENTATIVE: Michael Best & Friedrich, LLP; Frenchick, Grady J.; King, Karen B.
NUMBER OF CLAIMS: 29
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 1340
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods for **isolating** biological target materials, particularly nucleic acids, such as DNA or RNA or hybrid molecules of DNA and RNA, from other substances in a medium using silica magnetic particles. The methods of the present invention involve forming a complex of the silica magnetic particles and the biological target material in a mixture of the medium and particles, separating the complex from the mixture using external magnetic force, and eluting the biological target material from the complex. The preferred embodiments of magnetic silica particles used in the methods and kits of the present invention are capable of forming a complex with at least 2 .mu.g of biological target material per milligram of particle, and of releasing at least 60% of the material from the complex in the elution step of the method. The methods of the present invention produce **isolated** biological target material which is substantially free of contaminants, such as metals or macromolecular substances, which can interfere with further processing or analysis, if present.

TI Methods of **isolating** biological target materials using silica magnetic particles

AB The present invention provides methods for **isolating** biological target materials, particularly nucleic acids, such as DNA or RNA or hybrid molecules of DNA and RNA, from other. . . of the material from the complex in the elution step of the method. The methods of the present invention produce **isolated** biological target material which is substantially free of contaminants, such as metals or macromolecular substances, which can interfere with further. . .

SUMM The present invention relates to methods for separating or **isolating** a biological target material from other substances in a medium to produce an **isolated** material of sufficient purity for further processing or analysis. The present invention particularly relates to methods for separating or **isolating** biological target materials using magnetically responsive particles capable

reversibly binding the material. The present invention more specifically relates to methods for separating or **isolating** biological target materials using at least one magnetically responsive particle comprising silica or a silica derivative such as silica gel. . .

SUMM . . . include macromolecular substances from the in vivo or in vitro medium from which a nucleic acid material of interest is **isolated**, macromolecular substances such as enzymes, other types of proteins, polysaccharides, or polynucleotides, as well as lower molecular weight substances, such. . . inhibitors or oligonucleotides. Contaminants can also be introduced into a target biological material from chemicals or other materials used to **isolate** the material from other substances. Common contaminants of this last type include trace metals, dyes, and organic solvents.

SUMM . . . cells from body fluids such as blood, lymph, milk, urine, feces, semen, or the like, cells in culture, agarose or **polyacrylamide** gels, or solutions in which target nucleic acid amplification has been carried out, typically include significant quantities of contaminants from which the DNA or RNA of interest must be **isolated** before being used in a molecular biological procedure.

SUMM . . . Chapter 4 (RNA) of F. Ausubel et al., eds., Current Protocols in Molecular Biology, Wiley-Interscience, New York (1993). Conventional DNA **isolation** protocols generally entail suspending the cells in a solution and using enzymes and/or chemicals, gently to lyse the cells, thereby releasing the DNA contained within the cells into the resulting lysate solution. For **isolation** of RNA, the conventional lysis and solubilization procedures include measures for inhibition of ribonucleases and contaminants to be separated from. . .

SUMM Conventional nucleic acid **isolation** procedures have significant drawbacks. Among these drawbacks are the time required for the multiple processing steps necessary in the extractions. . .

SUMM . . . used effectively, and nucleic acids cannot be left in the presence of phenol. Generally also, multi-step procedures are required to **isolate** RNA after phenol/chloroform extraction. Ethanol (or isopropanol) precipitation must be employed to precipitate the DNA from a phenol/chloroform-extracted aqueous solution. . . single or double-stranded oligonucleotide contaminants from the DNA. Moreover, under the best circumstances such methods produce relatively low yields of **isolated** nucleic acid material and/or **isolated** nucleic acid material contaminated with impurities.

SUMM . . . in the art for methods, that are simpler, safer, or more effective than the traditional phenol/chloroform extraction/ethanol precipitation methods to **isolate** DNA and/or RNA sufficiently for manipulation using molecular biological procedures.

SUMM . . . from cells according to size is required for many molecular biological procedures. Such fractionation is typically accomplished by agarose or **polyacrylamide** gel electrophoresis. For analysis or treatment by a molecular biological procedure after fractionation, the DNA in the fraction(s) of interest must be separated from contaminants, such as agarose, other polysaccharides, **polyacrylamide**, **acrylamide**, or acrylic acid, in the gel used in such electrophoresis. Thus, there is also a need in the art for. . .

SUMM . . . such as water or an elution buffer. Numerous commercial sources offer silica-based matrices designed for use in centrifugation and/or filtration **isolation** systems. See, e.g. Wizard.TM. DNA purification systems line of products from Promega Corporation (Madison, Wis., U.S.A.); or the QiaPrep.TM. line of DNA **isolation** systems from Qiagen Corp. (Chatsworth, Calif., U.S.A.)

SUMM Magnetically responsive particles (hereinafter, "magnetic particles") have conventionally been used to **isolate** and purify polypeptide molecules such as proteins or antibodies. In recent years, however, magnetic particles and methods for using magnetic particles

have been developed for the **isolation** of nucleic acid materials. Several different types of magnetic particles designed for use in nucleic acid **isolation** are described in the literature, and many of those types of particles are available from commercial sources. Such magnetic particles. . .

SUMM The magnetic particles designed to bind nucleic acid materials indirectly are generally used to **isolate** a specific nucleic acid material, such as mRNA, according to the following basic **isolation** procedure. First, a medium containing a nucleic acid material is placed in contact with a label capable of binding to. . . responsive particle. Several different commercial sources are available for streptavidin magnetic particles and reagents designed to be used in mRNA **isolation** using biotinylated oligo-dT as described above. See, e.g. PolyATtract.RTM. Series 9600.TM. mRNA **Isolation** System from Promega Corporation; or the ProActive.TM. line of streptavidin coated microsphere particles from Bangs Laboratories (Carmel, Ind., U.S.A.). Magnetic particles and label systems have also been developed which are capable of indirectly binding and **isolating** other types of nucleic acids, such as double-stranded and single-stranded PCR templates. See, e.g. BioMag.TM. superparamagnetic particles from Advanced Magnetics,. . .

SUMM Indirect binding magnetic separation systems for nucleic acid **isolation** or separation all require at least three components, i.e. magnetic particles, a label, and a medium containing the nucleic acid. . . require different solution and/or temperature reaction conditions from one another. Each additional component or solution used in the nucleic acid **isolation** procedure adds to the risk of contamination of the **isolated** end product by nucleases, metals, and other deleterious substances.

SUMM A few types of magnetic particles have also been developed for use in the direct binding and **isolation** of biological materials, particularly nucleic acid. One such particle type is a magnetically responsive glass bead, preferably of a controlled. . .

SUMM A second type of magnetically responsive particles designed for use in direct binding and **isolation** of biological materials, particularly nucleic acid, are particles comprised of agarose embedded with smaller ferromagnetic particles and coated with glass.. . . is also difficult to produce such particles with a sufficiently uniform and concentrated magnetic capacity to ensure rapid and efficient **isolation** of nucleic acid materials bound thereto.

SUMM What is needed is a method for **isolating** biological entities, particularly nucleic acids, using a magnetically responsive particle capable of rapidly and efficiently directly **isolating** such entities sufficiently free of contaminants to be used in molecular biology procedures.

SUMM Briefly, in one aspect, the present invention comprises a method of **isolating** a biological target material from other materials in a medium by:

SUMM separating the biological target material from the complex by eluting the biological target material whereby the **isolated** biological target material is obtained.

SUMM In a further aspect, the present invention is a method of **isolating** a biological target material of interest from other materials in a medium using silica magnetic particles capable of reversibly binding. . . about 75% of the biological target material adhered to the silica magnetic particles is subsequently eluted. The biological target material **isolated** according to the method of this invention is preferably nucleic acid.

SUMM In another aspect, the present invention is a method of **isolating** plasmid DNA from other materials in a medium using a preferred form of silica magnetic particle, i.e., siliceous-oxide coated magnetic. . .

SUMM In a further aspect, the present invention is a kit for **isolating** a biological target material from a medium containing

the same, the kit comprising an aliquot of siliceous-oxide coated magnetic particles. . . 2 micrograms of the biological target material per milligram of particle. Optionally, the kit may include other components needed to **isolate** a biological target material from a medium containing the same according to the methods of the present invention.

SUMM . . . in the temperature range at which silica magnetic particles containing such materials are used according to the present methods to **isolate** biological materials.

SUMM . . . in the present invention preferably further comprise ferromagnetic material incorporated into a silica gel matrix. The elution step in the **isolation** methods of this invention are preferably accomplished without substantial contamination of the nucleic acid material by metal or metal compounds. . .

SUMM The present invention provides convenient and efficient means for **isolating** biological target material of interest from a variety of different media. A preferred aspect of the present method described briefly above, wherein magnetic force is used to remove the particles from the media, offers significant advantages over conventional **isolation** methods wherein a biological target material is reversibly bound to other silica material. Specifically, the magnetic removal step of the method substitutes for vacuum filtration or centrifugation steps required in conventional silica binding and elution **isolation** methods. It is, therefore, particularly amenable to being automated. Small laboratories or individual researchers frequently must purchase specialized and expensive. . .

SUMM The biological target material **isolated** using the **isolation** method of the present invention is sufficiently free of contaminating material for additional processing or analysis using standard molecular biology techniques. Applications of the present methods to **isolate** various different biological target materials from a variety of different media will become apparent from the detailed description of the. . .

DETD The biological target material **isolated** using the methods of the present invention is preferably a nucleic acid or a protein, more preferably a nucleic acid material such as RNA, DNA, or a RNA/DNA hybrid. When the biological target material **isolated** using the present methods is a nucleic acid, it is preferably DNA, or RNA including but not limited to plasmid. . . an amplification reaction such as the polymerase chain reaction (PCR), single-stranded DNA, mRNA, or total RNA. The nucleic acid material **isolated** according to the methods of the present invention is even more preferably a plasmid DNA or total RNA.

DETD Since nucleic acids are the most preferred biological target material **isolated** using the methods of the present invention, most of the detailed description of the invention below describes this preferred aspect. . . one of ordinary skill in the art of the present invention to use the methods of the present invention to **isolate** biological target materials other than nucleic acid materials, e.g., proteins or antibodies.

DETD The present methods of **isolating** biological target material can be practiced using any silica magnetic particle, but the methods are preferably practiced using the SOCM. . .

DETD The silica magnetic particles may contain substances, such as **transition metals** or volatile organics, which could adversely affect the utility of **isolated** biological target material substantially contaminated with such substances. Specifically, such contaminants could affect downstream processing, analysis, and/or use of the. . . present invention are preferably present in a form which does not readily leach out of the particle and into the **isolated** biological target material produced according to the

methods of the present invention. Iron is one such undesirable contaminant, particularly when. . . the accuracy of the results of quantitative spectrophotometric analysis of such samples. Any iron containing silica magnetic particles used to **isolate** nucleic acids using the present invention preferably do not produce **isolated** nucleic acid material sufficiently contaminated with iron for the iron to interfere with spectrophotometric analysis of the material at or. . .

DETD . . . more than 50 ppm, more preferably no more than 10 ppm, and most preferably no more than 5 ppm of **transition metals** when **assayed** as follows. Specifically, 0.33 g of the particles (oven dried @ 110.degree. C.) into 20 ml. of 1N HCl aqueous. . . mixture is then analyzed for metals content. Any conventional elemental analysis technique may be employed to quantify the amount of **transition metal** in the resulting liquid, but inductively coupled plasma spectroscopy (ICP) is preferred.

DETD The biological target material **isolated** using the method of the present invention can be obtained from eukaryotic or prokaryotic cells in culture or from cells. . . cosmetics; or any other source of cells. Some biological target materials, such as certain species of DNA or RNA are **isolated** according to the present method from the DNA or RNA of organelles, viruses, phages, plasmids, viroids or the like that. . . familiar to those in the art to obtain an aqueous solution of DNA or RNA, to which the separation or **isolation** methods of the invention are applied. The DNA or RNA, in such a solution, will typically be found with other. . .

DETD Regardless of the nature of the source of such material, the biological target material to be **isolated** in the present methods is provided in a medium comprising the biological target material and other species. The biological target. . . of the nucleic acid material to the silica magnetic particles. Therefore, in cases where the nucleic acid material to be **isolated** using the methods of the present invention is contained within a cell, the cell is preferably first processed by lysing. . .

DETD In a particularly preferred aspect of the present method, the nucleic acid material of interest **isolated** according to the method of the present invention is plasmid DNA initially contained in an E. coli bacteria cell. The. . .

DETD . . . using a magnetic field. Other forms of external force in addition to the magnetic field can also be used to **isolate** the biological target substance according to the methods of the present invention after the initial removal step. Suitable additional forms. . .

DETD The nucleic acid material eluted using the method of the present invention is suitable, without further **isolation**, for analysis or further processing by molecular biological procedures. The eluted nucleic acid can be analyzed by, for example, sequencing,. . .

DETD . . . or for expression of genes on the DNA which are capable of being expressed in the transformed host. Plasmid DNAs **isolated** by methods of the present invention have been found to be more efficiently transfected into eukaryotic cells than those **isolated** by the prior art method, wherein diatomaceous earth is employed in place of the silica gel in the methods of. . .

DETD The same batch of SOCM particles was used to produce the **assay** results presented in Examples 1 and 6 below, while a second batch of SOCM particles was used to generate the. . . ml/g for particles of >600 .ANG. diameter, median particle size of 5.3 .mu.m, and iron leach of 2.8 ppm when **assayed** as described herein above using ICP. The other batch of SOCM particles used in the Examples below were found to. . .

DETD **Assay** of Binding Capacity and Elution Efficiency of Silica Magnetic Particles for Plasmid DNA

DETD . . . from the total amount of plasmid added to the particles in each
sample, as follows. The liquid fraction of the **assay** mixture was separated from the magnetic silica by centrifugation at 14,000.times.g for 20 seconds. The amount of plasmid DNA remaining. . .

DETD . . . silica magnetic particles by adding 1 ml of DI water at room temperature. The particles were removed from the resulting **isolated** plasmid DNA solution by centrifugation. The amount of plasmid DNA eluted was then determined by measuring the absorbency of the. . .

DETD The overall efficiency of the plasmid **isolation** process was determined as the percent of DNA recovered in the final elution compared
to the amount of DNA incubated. . .

DETD The results of the binding **assay** described above are presented in FIG. 1, and together with the elution results in FIG. 3. The DNA binding capacity. . .

DETD The results of the elution **assay** described above are presented in FIG. 2, and together with the elution results in FIG. 3. The results show that. . .

DETD The results displayed in FIGS. 1-3 clearly demonstrate that the silica magnetic particles **assayed** herein exhibit excellent binding and elution characteristics.

DETD **Assay** of Binding Capacity and Elution Efficiency of Silica Magnetic Particles for DNA Fragments

DETD . . . digest was eluted by adding 200 .mu.l of DI water at room temperature. The particles were removed from the resulting **isolated** .lambda. digest solution by centrifugation. The amount of .lambda. Hind III digest DNA eluted was then determined by measuring the. . .

DETD Similar silica magnetic particle binding and elution **assays** were performed using .phi.X174 DNA digested with Hae III restriction enzyme, a digestion reaction which produces 10 DNA fragments ranging.
.

DETD . . . weights at different weights, the DNA fragments bound to and eluted from the silica magnetic particles in Example 2 were **assayed** using electrophoresis as follows. Samples of .lambda. Hind III digest eluted from two different samples of silica magnetic particles were. . . of the eluted DNA fragments from each of the restriction enzyme digests were compared to the control digests prior to
capture and elution on magnetic silica.

DETD FIGS. 4 and 5 show the visual image generated by the fluorometer from the fluorescent stained agarose gel of fractionated **captured** and eluted DNA fragments produced as described above. FIG. 4 shows 2 .mu.g of .lambda.HindIII digest electrophoesed on 1% agarose. . .

DETD . . . was noted between the control and samples from either set of digest samples analyzed herein, indicating the silica magnetic particles
assayed herein do not selectively bind or release DNA fragments according to molecular weight.

DETD **Isolation** of Plasmid DNA From Bacterial Cultures Using Silica Magnetic Particles and Magnetic Force

DETD Some of the resuspended silica magnetic particles prepared in Example 1 were used to **isolate** pGEM.RTM.-3zf(+) plasmid DNA from a culture of DH5.alpha. E. coli bacteria transformed with either form of plasmid DNA. The following solutions were used in the **isolation** procedure:

DETD Plasmid DNA was then **isolated** from the cleared lysate using the silica magnetic particles suspended in a solution of guanidine hydrochloride prepared in Example 1. Essentially the same procedure was used to **isolate** the plasmid DNA using the particles and magnetic force, as was used in the plasmid binding **assay** described in Example 2. However, the present **isolation**

procedure was initiated by adding 1 ml of the suspended silica magnetic particles to the cleared lysate produced from step. . . plasmid DNA. The volumes of each solution added to the magnetic silica particles at each subsequent step of the present **isolation** procedure followed were adjusted proportionately to account for the larger starting volume.

DETD The resulting **isolated** plasmid DNA was **assayed** qualitatively using gel electrophoresis, and quantitatively using a spectrophotometer. The gel **assay** results showed a high percentage of intact, supercoiled plasmid DNA present in the sample. The optical density measurements accurately reflected. . .

DETD **Isolation** of Plasmid DNA From Bacterial Cultures Using Silica Magnetic Particles and Vacuum Filtration

DETD . . . bacteria transformed with plasmid DNA, such as the cleared lysate production procedure used in Example 4. The plasmid is then **isolated** from the resulting cleared lysate using the suspension of silica magnetic particles of Example 1, but using vacuum filtration rather. . . particles. Vacuum filtration is also used to remove the wash solution from the particles in the washing steps of the **isolation** procedure.

DETD The resulting mixture was incubated for 5 minutes at room temperature, after which the particles were **captured**, using magnetic force to draw the particles to one side of the container while the supernatant was decanted into a. . .

DETD The **captured** particles in the first container were then washed three times with Column Wash Solution, prepared as described in Example 4, above. The particles were **captured** after each wash step, and the wash solution decanted. Each decanted wash solution was saved and counted. The sum of. . .

DETD After the third wash step, the RNA was eluted from the **captured** and washed particles by resuspending the particles in 250 .mu.l of Nanopure water heated to 37.degree. C., and then using. . .

DETD . . . in each experiment. FIG. 6 shows that of 200,000 CPMs of RNA exposed to the magnetic silica particles in this **assay**, an average of 125,000 CPMs became bound to the particles, and about 100,000 of the CPMs bound to the particles. . .

DETD These RNA binding and elution **assay** results are comparable to the DNA binding and elution results described in Example 2, above. The present **assay** shows the potential application of the magnetic silica particles according to the methods of the present invention to **isolate** RNA.

DETD No absorbance above background at 260 nm was observed in any of the wash solutions obtained by **assaying** the silica magnetic particles used in the Examples above.

CLM What is claimed is:

1. A method for **isolating** a biological target material from other material in a medium by: a. providing a medium including the biological target material;. . . magnetic field; and e. separating the biological target material from the complex by eluting the biological target material, whereby the **isolated** biological target material is obtained.

2. A method of **isolating** a biological target material according to claim 1, wherein the biological material **isolated** according to the method consists of a nucleic acid.

3. A method of **isolating** a biological target material according to claim 1, wherein the silica magnetic particles provided in step (b) are capable of. . .

4. A method of **isolating** a biological target material according to claim 3, wherein the silica magnetic particles provided in step (b) of the method. . .

5. A method of **isolating** a biological target material according to claim 1, wherein at least 60% of the biological target material in the complex. . .

6. A method of **isolating** a biological target material according to claim 1, wherein the biological target material eluted from the complex in step e. contains no more than 50 parts per million of **transition metal** contaminants.

7. A method of **isolating** a biological target material from other materials in a medium comprising the steps of: a) providing a medium containing the. . . material from the silica magnetic particle by exposing the particle to an elution solution; whereby the biological target material is **isolated**.

8. A method of **isolating** a biological target material according to claim 7, wherein the biological material **isolated** according to the method consists of a nucleic acid material.

9. A method of **isolating** a biological target material according to claim 8, wherein the nucleic acid biological target material **isolated** according to the method consists of a plasmid DNA material.

10. A method of **isolating** a biological target material according to claim 8, wherein the nucleic acid biological target material **isolated** consists of DNA fragment material.

11. A method of **isolating** a biological target material according to claim 7, wherein the silica magnetic particles provided in step (b) of the method. . .

12. A method of **isolating** a biological target material according to claim 7, wherein the mixture formed in step (c) comprises the medium, the silica. . .

13. A method of **isolating** a biological target material according to claim 12, wherein the chaotropic salt in the mixture formed in step (c) consists. . .

14. A method of **isolating** a biological target material according to claim 12, wherein the concentration of chaotropic salt in the mixture formed in step. . .

15. A method of **isolating** a biological target material according to claim 7, wherein the biological target material is adhered to the silica magnetic particle. . .

16. A method of **isolating** a biological target material according to claim 15, wherein the biological target material is adhered to the silica magnetic particle. . .

17. A method of **isolating** a biological target material according to claim 7, further comprising a step of washing the silica magnetic particle after removal. . .

18. A method of **isolating** a biological target material of claim 17, wherein the washing step is done using a wash solution comprising an alcohol. . .

19. A method of **isolating** a biological target material according to claim 18, wherein the washing step is done using a wash solution comprising at. . .

20. A method of **isolating** a biological target material according to claim 7, wherein the biological target material is eluted from the silica magnetic particle. . .

21. A method of **isolating** biological target material according to claim 7, wherein the biological target material eluted from the silica magnetic particle in step. . .

22. A method of **isolating** a plasmid DNA material from other materials in a medium comprising the steps of: a) providing a medium

containing the. . .
23. A method of **isolating** a plasmid DNA material according to claim 22, wherein the chaotropic salt in the mixture formed in step (c) is. . .
24. A method of **isolating** a plasmid DNA material according to claim 22, wherein the concentration of chaotropic salt in the mixture formed in step. . .
25. A method of **isolating** a plasmid DNA material according to claim 22, further comprising a step of washing the siliceous oxide-coated magnetic particle after. . .
26. A method of **isolating** a plasmid DNA material according to claim 25, wherein the washing step is done using a wash solution comprising an. . .
27. A method of **isolating** a plasmid DNA material according to claim 25, wherein the washing step is done using a wash solution comprising at. . .
28. A method of **isolating** a plasmid DNA material according to claim 22, wherein the plasmid DNA eluted from the silica magnetic particle in step. . .
29. A method of **isolating** a plasmid DNA material from other materials in a medium comprising the following steps, in the order indicated, of: a). . .

L19 ANSWER 37 OF 57 USPATFULL
ACCESSION NUMBER: 97:49558 USPATFULL
TITLE: Biomolecules bound to polymer or copolymer coated catalytic inorganic particles, **immunoassays** using the same and kits containing the same
INVENTOR(S): Kidwell, David A., Alexandria, VA, United States
Conyers, Susan M., Kalamazoo, MI, United States
PATENT ASSIGNEE(S): Geo-Centers, Inc., Newton Centre, MA, United States (U.S. corporation)
The United States of America as represented by the Secretary of the Navy, Washington, DC, United States (U.S. government)

	NUMBER	DATE
PATENT INFORMATION:	US 5637508	19970610
APPLICATION INFO.:	US 1995-376396	19950123 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1993-37981, filed on 26 Mar 1993, now patented, Pat. No. US 5384265, issued on 24 Jan 1995
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Chin, Christopher L.
LEGAL REPRESENTATIVE: Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 1055
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Polymer or copolymer coated catalytic colloidal metal particles bound to a biomolecule such as an antibody, avidin, or streptavidin and kits containing such polymer or copolymer coated catalytic metal particles are useful for detecting the presence of the biomolecule in an **assay** such as an **immunoassay**.

TI Biomolecules bound to polymer or copolymer coated catalytic inorganic particles, **immunoassays** using the same and kits containing the same

AB . . . containing such polymer or copolymer coated catalytic metal particles are useful for detecting the presence of the biomolecule in an **assay** such as an **immunoassay**.

SUMM The present invention relates to biomolecules which are bound to catalytic inorganic particles, **immunoassays** which utilize such biomolecules, and kits for carrying out such **immunoassays**.

SUMM . . . significant compounds, such as steroids or drugs of abuse, is often accomplished quickly and inexpensively by the employment of an **immunoassay**. Such an **assay** relies on an immunogenic recognition of the substance in question followed by the amplification of that recognition. Enzymes are widely used in **immunoassays** as the amplifier of the antibody-antigen recognition event. One of the most common types of **immunoassays** is the Enzyme-Linked Immunosorbant **Assay** (ELISA).

SUMM . . . may be preformed in a number of different ways. The two most common are the competitive mode and the sandwich **assay**. In a competitive mode ELISA, a surface, usually either a polystyrene plate or a nitrocellulose membrane, is coated with a **capture** antigen. These surfaces are normally chosen because they bind protein non-specifically. Therefore, if the antigen is not a protein, it. . .

SUMM In a sandwich **assay** ELISA, an antibody that recognizes part of the antigen is bound to a surface. Since antibodies are proteins, this is. . . test fluid is then added. If an antigen is present in the test fluid, the antibody on the surface will **capture** the antigen. Then a second, enzyme-labeled antibody, which recognizes a different part of the antigen than the first antibody, is added. The second antibody will then bind to the antigen which is **captured** on the surface. After washing the surface to remove any unbound materials, a substrate for the enzyme is added and. . . an ELISA, the signal is directly proportional to the concentration of the antigen in a test sample. Such a sandwich **assay** is widely used in the commercial arena for home pregnancy tests.

SUMM . . . light from some chemiluminescent reaction, is produced that can be observed macroscopically. Without this amplification step, the sensitivity of an **immunoassay** would be orders of magnitude less.

SUMM Several problems occur in the use of enzymes as amplifiers in **immunoassays**. They are:

SUMM 1. Any change in enzyme activity will affect the precision of the **assay**. For example, loss of half of the activity of the enzyme in a competitive ELISA may produce a false positive. . .

SUMM . . . $5000/g, a cost which is about 450 times the cost of gold. Fortunately, very little enzyme is necessary for each **assay**.

SUMM 4. Enzymes are often heterogeneous materials due to their **isolation** from natural sources. Therefore, characterization of enzyme-antibody conjugates can be difficult.

SUMM Colloidal metals have been employed in **immunoassays** previously. Mostly, they consisted of either colloidal iron or gold (M. Horisberger, "Colloidal Gold: A Cytochemical Marker for Light and. . . 19-40 (1981); and Martin et al, "Characterization of Antibody Labelled Colloidal Gold Particles and Their Applicability in a sol Particle **Immunoassay**, SPIA", J. **Immunoassay**, vol. 11, pp. 31-48 (1990)). However, in either case, the metals were only chosen for their color, i.e., their presence. . .

SUMM . . . However, the hydrogen peroxide is produced by photolysis with such compounds, and accordingly, the use of such labels in an **assay** requires the use of photolysis equipment.

SUMM Accordingly, it is an object of the present invention to provide novel compounds which can be used for the **immunoassay** of an analyte which do not suffer from the above-described drawbacks.

SUMM It is another object of the present invention to provide a novel **immunoassay** utilizing a compound in which a biomolecule is bound to a catalytically active moiety which is stable on long-term storage.

SUMM It is another object of the present invention to provide a novel **immunoassay** utilizing a compound in which a molecule is bound to a catalytically active moiety which is stable at elevated temperatures.

SUMM It is another object of the present invention to provide novel kits for carrying out such **immunoassays**.

SUMM . . . catalytic particles for the replacement of enzyme amplification of molecular recognition, their labeling with proteins, their purification, their use in **immunoassays**, and kits for carrying out such **immunoassays**.

DRWD FIG. 1 schematically illustrates a competitive mode **assay** according to the present invention;

DRWD FIG. 2, schematically illustrates a sandwich mode **assay** according to the present invention; and

DRWD FIG. 3 schematically illustrates a DNA **assay** according to the present invention;

DETD . . . comprise any metal which is catalytically active as a colloidal particle and meets those criteria. Preferably, the metal is any

transition metal. Particularly preferred metals are Ni, Fe, Ag, Pt, or Pd. Most preferably, the metal is Pt. Alloys of one or. . .

DETD . . . term "catalytically active" means the ability to catalyze any reaction which may conveniently used as the signal amplification in an **immunoassay**. Examples of such reactions include:

DETD . . . Analytical Chemistry USSR, vol. 28, pp. 1004-1008 (1971)). In no case were these particles attached to biomolecules or employed in **immunoassays**, and in most cases the reaction conditions employed are quite severe. All of the references cited in this and the. . .

DETD Some of the classical reducing agents produce colloidal particles that are too large for **immunoassays** or coagulate during formation. Thus, a large series of buffers and reducing conditions were tested as in Example 1. Some. . .

DETD . . . on an agarose gel, and the shift in mobility noted when the colloidal metal is bound to the biomolecule. Alternatively, **immunoassays** are run with the product and the concentration of the biomolecule is chosen to produce maximum sensitivity. The metal particles. . .

DETD . . . the context of the present invention, the term biomolecule refers to any molecule which can be used in an immunological **assay**. Specifically, biomolecules which may be used in the present invention included antibodies (monoclonal and polyclonal), avidin, and streptavidin, proteins, proteins. . .

DETD . . . present biomolecules which are bound to a colloidal metal particle may be used in a number of different types of **immunoassays**. The present biomolecules are particularly useful as replacements for the enzyme-linked biomolecules currently used in ELISAs.

DETD ELISAs are discussed in detail in Tijssen, Practice and Theory of Enzyme **Immunoassays**, Elsevier, N.Y., (1985), which is incorporated herein by reference. ELISAs are also discussed in Engvall et al, Immunochemistry, vol. 8,. . . 31,006; and Reissue Pat. No. 32,696; all of which are incorporated by reference. Enzymes which are used in activity amplification **assays** include peroxidase, .beta.-galactosidase, alkaline phosphatase, urease, glucose oxidase, glucoamylase, carbonic anhydrase, and acetylcholinesterase. As noted above, Horseradish peroxidase is the. . .

DETD . . . is used in conjunction with an antibody bound to biotin. In this way, it is possible to tailor the present **assay** for the detection of any desired antigen by judicious choice of the antibody bound to biotin.

DETD In a first type of DNA or RNA **assay**, a sequence of DNA or RNA which is complementary to the target DNA or RNA is immobilized on a support,. . .

DETD . . . particularly useful for detecting the presence or absence of a target DNA in conjunction with PCR amplification. This type of **assay** is schematically illustrated in FIG. 3. A sample which may contain the target DNA (6) is incubated in steps (a). . .

DETD . . . streptavidin (11) and, thus, detected after washing. It is noteworthy that enzymes are not suitable as labels for such an **assay** because they will be denatured by the heating step required by PCR.

DETD In the **assays** utilizing DNA or RNA labelled with a colloidal metal particle, the colloidal metal particle will typically be linked to the. . .

DETD In another embodiment, the present invention relates to kits for carrying out an **assay** utilizing a biomolecule bonded to a catalytically active colloidal metal according to the present invention. Such kits will typically contain. . . a second container containing a premeasured and known amount of the target analyte to serve as a standard for the **assay**. The kit may further contain written

instructions for carrying out the **assay**. The biomolecule bound to the colloidal metal may be contained in the kit in the form of a dry powder. . .

DETD . . . particles, then three bands containing platinum appear, two of which are unlabeled platinum. Similar patterns are observed on native, gradient **polyacrylamide** gels.

DETD . . . retained by a 20 nm Anotop membrane. The retained material may be removed from the membrane and employed in an **immunoassay**. This is an alternative method to using a sizing column for the removal of unbound protein.

DETD . . . amount of strepavidin was optimized by both observing the pattern on Agarose gels and the sensitivity produced in a trial **immunoassay**. Either antibodies or BSA, the carrier protein, labeled with biotin were serially diluted in PBS and spotted onto nitrocellulose membranes.. . .

DETD . . . system produces a water-insoluble dye that localizes at the site of catalytic activity. The former system is for ELISA plate **assays** where a water-soluble dye is desirable.

DETD The reactions were also **assayed** photographically. Trials were run in ELISA plates that had eight rows of twelve 2000 .mu.l wells. The outside of the. . .

DETD These reactions were also **assayed** on nitrocellulose strips about three centimeters in length and one centimeter in width. Several platinum colloid dilutions were made in. . .

CLM What is claimed is:

8. The method of claim 1, which is a competitive **assay**.

9. The method of claim 1, which is a sandwich **assay**.

15. The kit of claim 11, further comprising written instructions for carrying out an **assay**.

L24 ANSWER 3 OF 6 USPATFULL
ACCESSION NUMBER: 1999:15680 USPATFULL
TITLE: Method for performing Rubella assay
INVENTOR(S): Jou, Yi-Her, Libertyville, IL, United States
Stroupe, Stephen D., Libertyville, IL, United States
Adamczyk, Janina, Gurnee, IL, United States
Safford, John, Libertyville, IL, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5866322	19990202
APPLICATION INFO.:	US 1991-776495	19911011 (7)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1989-375029, filed on 7 Jul 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-150278, filed on 29 Jan 1988, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Green, Lora M.
LEGAL REPRESENTATIVE: Weinstock, Steven F.
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
LINE COUNT: 3352
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes novel rubella **assays** employing a Rubella virus **capture** reagent and a solid phase material containing a reaction site comprising a polymeric cation substance. A test sample suspected of containing Rubella antibody may be contacted with the **capture** reagent to form a **capture** reagent/analyte complex. The complex is then contacted to the positively charged solid phase to attract, attach, and immobilize the **capture** reagent/analyte complex.
AB The present invention includes novel rubella **assays** employing a Rubella virus **capture** reagent and a solid phase material containing a reaction site comprising a polymeric cation substance. A test sample suspected of containing Rubella antibody may be contacted with the **capture** reagent to form a **capture** reagent/analyte complex. The complex is then contacted to the positively charged solid phase to attract, attach, and immobilize the **capture** reagent/analyte complex.
SUMM This invention relates generally to the field of binding **assay** devices and methods. In particular, the present invention relates to novel methods and products useful in the performance of a rubella **immunoassay**.
SUMM Various analytical procedures and devices are commonly employed in **assays** to determine the presence and/or concentration of substances of interest or clinical significance which may be present in biological liquids. . .
SUMM **Immunoassay** techniques take advantage of the mechanisms of the immune systems of higher organisms, wherein antibodies are produced in response to. . .
SUMM There are several known **immunoassay** methods using immunoreactants, wherein at least one of the immunoreactants is labeled with a detectable component so as to be. . . one labeled antibody and an unlabeled antibody bound to a solid phase support such that the

complex can readily be **isolated**. In this example, the amount of labeled antibody associated with the solid phase is directly proportional to the amount of. . .

SUMM An alternative technique is the "competitive" **assay**. In one example of a competitive **assay**, the **capture** mechanism again may use an antibody attached to an insoluble solid phase, but a labeled analyte (rather than a labeled. . . immobilized antibody. Similarly, an immobilized analyte can compete with the analyte of interest for a labeled antibody. In these competitive **assays**, the quantity of **captured** labeled reagent is inversely proportional to the amount of analyte present in the sample.

SUMM Despite their great utility, there are disadvantages with such **assay** methods. First, the heterogenous reaction mixture of liquid test sample and soluble and insoluble **assay** reagents, can retard the kinetics of the reaction. In comparison to a liquid phase reaction wherein all reagents are soluble,. . . referred to as nonspecific binding and can interfere with the detection of a positive result. Third, with conventional immobilization methods, **separate** batches of manufactured solid phase reagents can contain variable amounts of immobilized binding member.

SUMM With regard to the manufacture of solid phase devices for use in binding **assays**, there are a number of **assay** devices and procedures wherein the presence of an analyte is indicated by the analyte's binding to a labeled reagent and/or. . .

SUMM The use of porous teststrips in the performance of specific binding **assays** is also well-known. In a sandwich **assay** procedure, a test sample is applied to one portion of the teststrip and is allowed to migrate through the strip. . . a component of the fluid test sample or with the aid of an eluting or chromatographic solvent which can be **separately** added to the strip. The analyte is thereby transported into a detection zone on the teststrip wherein an analyte-specific binding. . . the aid of a labeled analyte-specific binding member which may be incorporated in the teststrip or which may be applied **separately** to the strip.

SUMM . . . a solution by capillary action. Different areas or zones in the strip contain the reagents needed to perform a binding **assay** and to produce a detectable signal as the analyte is transported to or through such zones. The device is suited for chemical **assays** as well as binding **assays** which are typified by the binding reaction between an antigen and a complementary antibody.

SUMM . . . an immunosorbing zone, containing an immobilized specific binding member. The test sample is applied to the immunosorbing zone, and the **assay** result is read at the immunosorbing zone.

SUMM Alternative **separation** methods include the use of a magnetic solid phase, polymerization techniques and the formation of analyte complexes having characteristics different than the non-complexed analyte. Ullman et al. (U.S. Pat. No. 4,935,147) describe a method for **separating** charged suspended non-magnetic particles from a liquid medium by contacting the particles with charged magnetic particles and a chemical reagent.. . .

SUMM Longoria et al. (U.S. Pat. No. 4,948,726) describe an **assay** method involving the reaction of antigen and antibody molecules to form an antigen/antibody complex that uniquely exhibits an ionic charge. . . is then chosen for its unique affinity for the antigen/antibody complex. Milburn et al. (U.S. Pat. No. 4,959,303) describe an **assay** wherein antigen from a test sample and an antibody specific for the antigen are incubated under conditions sufficient for the. . . method of immobilizing the analyte molecule itself directly upon a solid support. Del Campo (U.S. Pat. No. 4,990,442) describes an **assay** involving the binding of the analyte itself directly to an amphillic support by hydrogen bonding. Lyle et al. (European

Application,. . . preferential immobilization of polynucleotides over
oligonucleotides to a polycationic support. Pronovost et al. (European Application, Publication No. 363,109) describe the **separation** of chlamydial or gonococcal antigen from a specimen using a positively charged solid support.

SUMM Vandekerckhove (U.S. Pat. No. 4,839,231) describes a two-stage, protein immobilization process involving an initial **separation** or **isolation** of **target** proteins in a gel, such as a polyacrylamide electrophoresis gel, followed by the transfer of those **isolated** proteins to the surface of a coated support for immobilization. The coated support is prepared by contacting a chemically inert. . .

SUMM . . . teststrip field. There is a growing demand for devices that require few or no manipulative steps to perform the desired **assay**, for devices that can be used by relatively untrained personnel, and for devices that provide results which are minimally affected by variations in the manner in which the **assay** is performed. Further considerations are the ease with which the resultant detection signal may be observed as well as the. . . addition, a device manufacturing format has long been sought which will enable the production of a "generic" device, i.e., an **assay** device for which the capacity of use is defined by the reagents used in the performance of the **assay** rather than the reagents used in the manufacture of the device.

SUMM The present invention provides novel a **assay** method for determining the presence or amount of rubella antibody in a test sample.

In one embodiment, the **assay** involves a **capture** reagent, a **capture** reagent, comprising a Rubella virus; an indicator reagent, comprising a specific binding member for Rubella antibody and a detectable label;. . . polymeric cation substance thereby imparting a net positive charge to the solid phase. The solid phase is contacted with the **capture** reagent and the test sample, whereby the polymeric cation of the solid phase attracts and attaches to the Rubella virus **capture** reagent, thereby immobilizing the **capture** reagent and complexes thereof. The solid phase is then contacted with the indicator reagent, thereby immobilizing the indicator reagent on. . .

SUMM The present invention also enables the production of a generic solid phase device for use in specific binding **assays**. **Assay** procedures for many different analytes can use the same solid phase material which contains a predetermined zone of cationic **capture** polymer rather than an immobilized binding member capable of binding only a specific analyte as found in conventional flow-through and. . .

SUMM The present invention provides two major advancements to the field of specific binding **assays**: a) the use of liquid phase kinetics facilitates the formation of a complex from the homogeneous mixture of analyte and **assay** reagent specific binding members, and b) the ion-**capture** technique increases the potential number of complexes that can be immobilized on a solid support. If the advantages of liquid. . .

SUMM The **assay** methods and reagents of the present invention can be used in a variety of **immunoassay** formats. The present invention, however, is not limited to immunoreactive **assays**. Any **assays** using specific binding reactions between the analyte and **assay** reagents can be performed.

SUMM The present invention is particularly directed to the performance of a binding **assay** for the detection of rubella antibody present in a test sample. The **assay** is based upon the unexpected discovery that the Rubella virus can be preferentially **captured** on a polycation-treated solid phase material and **separated** from free binding members present in the test solution, thereby allowing
the use of the Rubella virus as an ion-**capture** reagent in

binding **assays** for anti-Rubella antibody.

SUMM The **assay** reagents can include any suitable solid phase material containing a cationic **capturing** zone, Rubella viruses and an indicator agent, such as labeled anti-human IgG or IgM antibodies. Following the incubation of the test sample and the Rubella viruses, the test solution is contacted to the cationic **capturing** zone wherein the reaction between the polycationic substance and the Rubella viruses results in the **capture** of the Rubella viruses upon the solid phase material. Thus, the binding of the Rubella antibodies in the test sample. . .

SUMM . . . specific binding pairs are exemplified by the following: biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences (including probe and **capture** nucleic acid sequences used in DNA hybridization **assays** to detect a target nucleic acid sequence), complementary peptide sequences (including those formed by recombinant methods), effector and receptor molecules,. . .

SUMM . . . pretreated prior to use, such as preparing plasma from blood, diluting viscous liquids, etc. Methods of pretreatment can also involve **separation**, filtration, distillation, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples such as. . .

SUMM The term "analyte", as used herein, refers to the substance to be detected in or **separated** from the test sample by means of the present invention. The analyte can be any substance for which there exists. . .

SUMM The term "signal producing component", as used herein, refers to any substance capable of reacting with the analyte or another **assay** reagent to produce a reaction product or signal that indicates the presence or amount of the analyte and that is detectable by visual or instrumental means. "Signal production system", as used herein, refers to the group of **assay** reagents that are used to produce the desired reaction product or signal. For example, one or more signal producing components. . .

SUMM . . . the amount of an analyte in the test sample. Generally, the indicator reagent is detected or measured after it is **captured** on the solid phase material, but the unbound indicator reagent can also be measured to determine the result of an **assay**.

SUMM The specific binding member of the indicator reagent is capable of binding either to the analyte as in a sandwich **assay**, to the **capture** reagent as in a competitive **assay**, or to an ancillary specific binding member to complete a detectable complex. The label, as described above, enables the indicator. . . reagent enables the indirect binding of the label to the analyte, to an ancillary specific binding member or to the **capture** reagent. The selection of a particular label is not critical, but the label will be capable of generating a detectable. . .

SUMM As mentioned above, the label can become attached to the specific binding member during the course of the **assay**. For example, a biotinylated anti-analyte antibody may be reacted with a labeled streptavidin molecule. Any suitable combination of binding members. . .

SUMM The term "**capture** reagent", as used herein, refers to an unlabeled specific binding member which is attached to a charged substance. The attachment. . . attachment to the charged substance does not interfere with the binding member's binding site. The binding member component of the **capture** reagent is specific either for the analyte as in a sandwich **assay**, for the indicator reagent or analyte as in a competitive **assay**, or for an ancillary specific binding member, which itself is specific for the analyte.

SUMM The charged substance component of the **capture** reagent can include anionic and cationic monomers or polymers. For example, anionic polymers include polyglutamic acid (PGA), anionic protein or. . . member can be joined to more than one charged monomer or polymer to increase the net charge associated with the **capture** reagent.

SUMM The novel **capture** reagents of the present invention are used to facilitate the observation of the detectable signal by substantially **separating** the analyte and/or the indicator reagent from other **assay** reagents and the remaining test sample components. In its most advantageous use, the **capture** reagent is reacted with the test sample and **assay** reagents in a homogeneous reaction mixture. Following the formation of the desired specific binding member complexes, the complexes involving a **capture** reagent are removed from the homogeneous reaction mixture by contacting the homogeneous reaction mixture to a solid phase that is oppositely charged with respect to the charge of the **capture** reagent.

SUMM In one embodiment of the present invention, a negatively charged **capture** reagent can be prepared by conjugating the selected specific binding member to one or more activated polymeric anionic molecules and. . .

SUMM Typically, the negatively charged **capture** reagents of the following Examples were formed by reacting the desired specific binding member with an activated PGA molecule having. . .

SUMM . . . used to "activate" a specific binding member or polymeric anionic molecule, i.e., to prepare the specific binding member or the **polymeric** anionic molecule for chemical **cross-linking**. Activating agents also include thiol introducing agents such as the thiolanes (such as 2-iminothiolane), succinimidyl mercaptoacetates (such as N-succinimidyl-S-acetylmercaptoacetate), and. . .

SUMM . . . preferred agents for use with the particular polymeric anionic molecule and specific binding member to be used in the diagnostic **assay**. Therefore, it will be appreciated by those skilled-in-the-art that the coupling agent or activating agent used in a given **assay** will generally be determined empirically.

SUMM An example of the preparation of a negatively charged **capture** reagent involves the reaction of a specific binding member (SBM) having an amino group and an activated PGA having an. . .

SUMM In yet another embodiment of the present invention, a preferred anionic polymer for use in the **capture** reagent is carboxymethylamylose (CMA) due to its particular performance in various **immunoassay** configurations. The improved performance of **capture** reagents containing CMA can be attributed to the higher avidity of the CMA **capture** reagent for the cationic solid phase. This attribute is particularly advantageous in a two step sandwich **assay** format wherein a polyanion is used to block nonspecific binding of the indicator reagent to the cationi solid phase.

SUMM . . . specific binding member", as used herein, refers to any member of a specific binding pair which is used in the **assay** in addition to the specific binding members of the **capture** reagent and the indicator reagent. For example, in an **assay** an ancillary specific binding member may bind the analyte to a second specific binding member to which the analyte itself could not attach, or as in an inhibition **assay** the ancillary specific binding member may be a reference binding member. One or more ancillary specific binding members can be used in an **assay**.

SUMM . . . insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic charge and ability to attract the **capture** reagent, e.g., methylated wool, nylons, and special glasses having a positive charge. Alternatively, the solid phase can be pretreated with and retain a charged substance that is oppositely charged with respect to the charged substance of the **capture** reagent. For example, an anionic substance can be bound to a specific binding member to form the **capture** reagent, and a cationic substance can be applied to and retained by the solid phase, or vice versa.

SUMM . . . about 2% (exclusive of counter ion) are particularly

advantageous in preparing a solid phase that will undergo washing during the **assay** process. The use of such a polycationic substance to prepare a suitably charged solid phase resulted in a solid phase. . . could be subjected to a greater degree of manipulation without losing the capability to attract and retain the oppositely charged **capture** reagent. It was determined that polycationic substances having a nitrogen content above about 5% (exclusive of counter ion) were more. . .

SUMM An **assay** device based on the ion-**capture** technique can have many configurations, several of which are dependent upon the material chosen as the solid phase. In various. . .

SUMM The novel ion-**capture** devices of the present invention involve a solid phase made of any suitable porous material. By "porous" is meant that. . . phase materials. For example, the solid phase can include a fiberglass, cellulose, or nylon pad for use in a flow-through **assay** device having one or more layers containing one or more of the **assay** reagents; a dipstick for a dip and read **assay**; a teststrip for wicking or capillary action (e.g., paper, nitrocellulose, polyethylene) techniques; or other porous or open pore materials well-known. . .

SUMM . . . cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon); porous gels such as silica gel, agarose, dextran, and gelatin; **polymeric** films such as **polyacrylamide**; and the like. The solid phase should have reasonable strength or strength can be provided by means of a support,. . .

SUMM Preferred solid phase materials for flow-through **assay** devices include filter paper such as a porous fiberglass material or other fiber matrix materials as well as isotropically porous. . . the material used will be a matter of choice, largely based upon the properties of the sample or analyte being **assayed**, e.g., the fluidity of the test sample.

SUMM Typically, the novel teststrip and flow-through devices employing the ion-**capture** principles of the present invention are characterized by having the analyte, test sample and/or eluting solvent migrate through the device. . .

SUMM Uses for Ion-**Capture** Reagents

SUMM In accordance with the disclosure of the present invention, a sandwich **assay** can be performed wherein the **capture** reagent involves an analyte-specific binding member which has been bound to a charged substance such as an anionic polymer. The **capture** reagent is contacted with a test sample, suspected of containing the analyte, and an indicator reagent comprising a labeled analyte-specific. . .

SUMM In the exemplary sandwich **assay**, a binding reaction results in the formation of a **capture** reagent/analyte/indicator reagent complex. The resultant complex is then removed from the excess **assay** reagents and test sample of the homogenous reaction mixture by means of a solid phase that is either inherently oppositely charged with respect to the **capture** reagent or that retains an oppositely charged substance, for example a cationic polymer. In the ion-**capture assays**, the oppositely charged solid phase attracts and attaches to the **capture** reagent/analyte/indicator reagent complex through the interaction of the anionic and cationic polymers. The complex retained on the solid phase is. . . solid phase is proportional to the amount of analyte in the sample. The only major limitation inherent in the sandwich **assay** is the requirement for the analyte to have a sufficient size and appropriately orientated epitopes to permit the binding of at least two specific binding members. Other sandwich **assays** may involve

one or more ancillary specific binding members to bind the analyte to the indicator reagent and/or **capture** reagent.

SUMM The present invention also can be used to conduct a competitive **assay**. In an exemplary competitive **assay**, the soluble **capture** reagent again includes a specific binding member which has been attached to a charged substance, such as an anionic polymer. The **capture** reagent is contacted, either sequentially or simultaneously, with the test sample and an indicator reagent that includes a second binding member which has been labeled with a signal generating compound. Either the **capture** reagent and analyte can compete in binding to the indicator reagent (e.g., the **capture** reagent and analyte are antigens competing for a labeled antibody), or the indicator reagent and analyte can compete in binding to the **capture** reagent (e.g., the indicator reagent is a labeled antigen which competes with the antigen analyte for binding to the antibody component of the **capture** reagent). A competitive binding or displacement reaction occurs in the homogeneous mixture and results in the formation of **capture** reagent/analyte complexes and **capture** reagent/indicator reagent complexes.

SUMM The resultant complexes are removed from the excess **assay** reagents and test sample by contacting the reaction mixture with the oppositely charged solid phase. The **capture** reagent complexes are retained on the solid phase through the interaction of the oppositely charged polymers. The complexes retained on the solid phase can be detected via the label of the indicator reagent. In the competitive **assay**, the amount of label that becomes associated with the solid phase is inversely proportional to the amount of analyte in the sample. Thus, a positive test sample will generate a negative signal. The competitive **assay** is advantageously used to determine the presence of small molecule analytes, such as small peptides or haptens, which have a single epitope with which to bind a specific binding partner. Other competitive **assays** may involve one or more ancillary specific binding members to bind the analyte to the indicator reagent and/or **capture** reagent.

SUMM For example, in an **assay** for theophylline, an anti-theophylline antibody (either monoclonal or polyclonal) can be conjugated with an anionic polymer to form a soluble **capture** reagent, and a competition for binding to that antibody can be established between labeled theophylline (i.e., indicator reagent) and the. . . to a solid phase which retains a cationic polymer coating. The attraction between the oppositely charged ionic species of the **capture** reagent and the solid phase serves to **separate** the immunocomplex from the reaction mixture. The signal from the indicator reagent can then be detected. In this example, increased. . .

SUMM In addition, the present invention can be used in an inhibition **assay**, such as the measurement of an antibody by inhibiting the detection of a reference antigen. For example, the **capture** reagent can include an antibody/anionic polymer conjugate and the indicator reagent can be a labeled antibody. The test sample, suspected of containing an antibody analyte, is mixed with a reference antigen with which the **capture** reagent and indicator reagent can form a detectable sandwich complex that can be immobilized upon the solid phase by the ion-**capture** reaction. The degree of inhibition of antigen uptake by the **capture** reagent is proportional to the amount of antibody analyte in the test sample, thus, as the concentration of the antibody. . .

SUMM In general, once complex formation occurs between the analyte and the **assay** reagents, the oppositely charged solid phase is used as a **separation** mechanism: the homogeneous reaction mixture is contacted with the solid phase, and the newly formed binding complexes are retained on the solid phase through the interaction of the opposite charges of the solid phase and the **capture** reagent. If the user is not concerned with liquid phase kinetics, the **capture** reagent can be pre-immobilized on the solid phase to form a **capture** site.

SUMM The present invention can also be used for **separating** a substance from a liquid sample. For example, the **capture** reagent and solid phase can be used without an indicator reagent for the sole purpose of **separating** an analyte from a test sample. Furthermore, the **capture** reagent can be contacted with a soluble second charged substance which is oppositely charged with respect to the **capture** reagent. The second charged substance is not retained on the solid phase prior to contacting the sample to the solid phase material, but it attracts and attaches to the **capture** reagent such that the resultant **assay** complexes are retained on an oppositely charged solid phase.

SUMM When the complex of charged **capture** reagent and analyte (and/or indicator reagent) is contacted to the oppositely charged solid phase, the ionic attraction of the oppositely charged species governs the efficiency of the **separation** of the complex from the reaction mixture. The ionic attraction can be selected to provide a greater attraction than the immunological attraction of antibody for antigen, particularly when multiple polycationic and polyanionic species are included in the **capture** reagent and oppositely charged solid phase. A further advantage is that the "ion-**capture**" technique minimizes the nonspecific adsorption of interfering substances onto the solid phase, thereby offering improved accuracy of analysis. The ion-**capture** technique thereby enables the performance of an **assay** having a highly specific **separation** method, minimal nonspecific binding, and high sensitivity.

SUMM . . . to noise ratio. It was unexpectedly discovered that the nonspecific binding blocker could be a free polyanion even when the **capture** reagent used in the **assay** involved a polyanionic substance conjugated to a specific binding member. It would have been expected that the presence of a free or unbound polyanion would prevent, or at least reduce, the immobilization of the **capture** reagent on the solid phase. It was found, however, that the nonspecific blocker was more effective in inhibiting the direct, nonspecific binding of indicator reagent to the solid phase than it was in reducing the attachment of the polyanionic **capture** reagent to the polycationic solid phase. Suitable nonspecific binding blockers include, but are not limited to, dextran sulfate, heparin, carboxymethyl. . .

SUMM . . . nonspecific binding blocker added to the indicator reagent could be greater than the amount of polyanionic substance contained in the **capture** reagent. It was found that free polyanionic nonspecific binding blocker could be added to the indicator reagent in amounts 40,000 times the amount of polyanionic substance used in the **capture** reagent. Generally, the preferred amount of polyanionic blocker added to the indicator reagent is 50 to 14,000 times the amount of polyanionic substance used in the **capture** reagent. For two step sandwich **assays**, the preferred amount of polyanionic blocker added to the indicator reagent is 1000 to 2000 times that contained in the **capture** reagent.

SUMM An appropriate range of use can be determined for each analyte of interest. For example, in an **assay** to detect thyroid stimulating hormone (TSH) wherein dextran sulfate was added to the indicator reagent as a free polyanionic nonspecific binding blocker, suitable amounts of free polyanion ranged from 233 to 19,000 times that of the **capture** reagent, or about 0.1-8% dextran sulfate. As illustrated in the following Table, the preferred nonspecific binding blocker as well as. . .

SUMM

Nonspecific Binding Blocker in the Indicator Reagent		
Analyte	Preferred	More Preferred

% Dextran sulfate (MW 5,000)		
(blocker/**capture** reagent, w/w)		
TSH	0.1-8	0.5-2
	(233-19,000)	(1,000-4,000)
T3	0.1-2	0.1-0.2
	(2,000-40,000)	
		(2,000-4,000)
% Carboxymethyl cellulose (MW 250,000)		
(blocker/**capture** reagent, w/w)		
hCG	0.01-0.25	0.025
	(0.44-11)	(1.1)
HIV	0-0.2	0.05
	(0-20,000)	(5,000)

SUMM Moreover, it was discovered that the polyanionic nonspecific binding blocker could be added to the **assay** as a **separate** reagent, or it could be included as free polyanion in the **capture** reagent, in an ancillary binding member reagent, in a buffer reagent or in some other reagent used in the **assay**. For example, when free polyanion is included in the **capture** reagent, it can enhance the signal to noise ratio by neutralizing interfering materials which are contained either in the test sample itself or in the other **assay** reagents, or those which were introduced during the device manufacturing process. The following Table illustrates some preferred amounts of nonspecific binding blocker for different analytes of interest, wherein the free polyanion is contained in the **capture** reagent itself.

SUMM

Nonspecific Binding Blocker in the **Capture** Reagent Analyte	Preferred	More Preferred
% Dextran sulfate (MW 5,000)		
(blocker/**capture** reagent, w/w)		
Digoxin	0-0.004	0.004
	(0-222)	(222)
T3	0.004-0.01	0.004
	(66-165)	(66)

SUMM Depending upon the the analyte of interest and the desired **assay** configuration, the preferred nonspecific binding blocker, as well as the optimization of its concentration and whether it is included as a component of another **assay** reagent, is selected by empirical techniques which can be performed without undue experimentation by one of ordinary skill in the art of binding **assays**. In only one known instance, i.e., the use of 0.005% dextran sulfate in the **capture** reagent of a competitive digoxin **assay**, was there an inhibition of the binding between the **capture** reagent and solid phase due to the addition of the nonspecific binding blocker.

SUMM Ion-**capture Assay** Devices

SUMM As described above, ion-**capture assay** devices may include impermeable solid phase materials such as glass slides, magnetic particles, test tubes and plastic wells. However, it has also been discovered that the entire ion-**capture assay** can be performed in a porous solid phase material. The ion-**capture assay** devices of the present invention specifically involve any suitably absorbent, adsorbent, imbibing, bibulous, non-bibulous, isotropic or capillary possessing material (i.e.,. . .

SUMM Possible **assay** devices include, but are not limited to, a conventional chromatographic column, an elongated strip of porous material wherein the fluid. . .

SUMM . . . application site. In yet other alternative devices and methods, the indicator reagent can be added to the device as a **separate**

reagent solution, either sequentially or simultaneously with the test sample and/or **capture** reagent.

SUMM . . . to allow the analyte to migrate from one material to another. The different materials may contain different diffusive or immobilized **assay** reagents, with the individual material being assembled into an elongated strip or flow through pad device. In yet a further.

. two or more zones of the device may overlap. For example, the sample application zone may also contain a diffusive **assay** reagent (e.g., indicator reagent, **capture** reagent, etc.) which reacts with the analyte to form a complex or reactive product which continues to migrate to other zones in or on the device. In a further example, the

sample application zone may contain an immobilized **assay** reagent (e.g., polymer oppositely charged with respect to the **capture** reagent) which immobilizes the **capture** reagent or **capture** reagent complexes for detection. Again, those skilled-in-the-art will readily appreciate the applicability of the present invention to a variety of device formats wherein the indicator reagent is immobilized by directly or indirectly binding to a **capture** reagent conjugate that is in turn immobilized by an oppositely charged solid phase material.

SUMM . . . material. Fluid flow contact can include physical contact of the application pad to the porous material as well as the **separation** of the pad from the porous material by an intervening space or additional material which still allows fluid flow between. . .

SUMM . . . polyethylene pads and glass fiber filter paper. The material must also be chosen for its compatibility with the analyte and **assay** reagents, for example, glass fiber filter paper was found to be the preferred application pad material for use in a human chorionic gonadotropin (hCG) **assay** device.

SUMM In addition, the application pad may contain one or more **assay** reagents either diffusively or non-diffusively attached thereto. Reagents which can be contained in the application pad include, but are not. . . specific binding members, test sample pretreatment reagents and signal producing system components. For example, in a preferred embodiment of an ion-**capture** device an indicator reagent is predeposited in the application pad during manufacture; this eliminates the need to combine test sample and indicator reagent prior to using the

device. The **isolation** of **assay** reagents in the application pad also keeps interactive reagents **separate** and facilitates the manufacturing process. For example, the indicator reagent may be retained in the application pad in a dry. . . at a detection zone, and that indicator reagent which does not become immobilized at the detection zone due to the **assay** reaction will pass from the detection zone.

SUMM In a preferred ion-**capture** device, the application pad receives the test sample, and the wetting of the application pad by the test sample will. . . may serve a third function as both an initial mixing site and a reaction site for the test sample and **assay** reagent.

SUMM In another preferred embodiment, the application pad contains both the indicator reagent and the **capture** reagent in a dried form. The addition of the test sample reconstitutes the **assay** reagents, thereby enabling their reaction with the analyte and the formation of a charged indicator reagent/analyte/**capture** reagent complex. The complex then migrates from the application pad to the porous teststrip material for subsequent reaction with a polymeric material immobilized in a detection zone, wherein that polymeric material is oppositely charged with respect to the **capture** reagent. Alternatively, either the indicator reagent or the **capture** reagent may be contained in the porous teststrip material between the application pad and the detection zone. Preferably, the **capture** reagent complex is allowed to form prior to or concurrent with the migration of

the **capture** reagent into the detection zone.

SUMM . . . pad. The addition of test sample to the overcoated application pad causes the gelatin to dissolve, thereby rehydrating the predeposited **assay** reagent. In an alternative embodiment of the present invention, the reagent containing application pad is dried or lyophilized to increase. . .

SUMM In another preferred embodiment, the **assay** devices of the present invention can be further modified by the addition of a filtration means. The filtration means can be a **separate** material placed above the application pad or between the application pad and the porous material. Alternatively, the application pad material. .

.

SUMM . . . reaction of the test sample and the reagent(s) in the application pad. Alternatively, such a layer can contain an additional **assay** reagent(s) which is preferably **isolated** from the application pad reagents until the test sample is added. The flow control layer may also serve to prevent unreacted **assay** reagents from passing to the porous material.

SUMM The porous material used in the novel ion-**capture** devices of the present invention may be any suitably absorbant, porous or capillary possessing material through which a solution containing. . . of the optional application pad should be chosen for its ability to premix the test sample and one or more **assay** reagents: fluid flow through a nitrocellulose membrane is laminar and does not provide the more turbulent flow characteristics which allow. . . fiber filter paper are appropriately used as application pads to enable the mixing and reaction of the test sample and **assay** reagents within the application pad. An especially preferred porous material is glass fiber filter paper.

SUMM . . . the porous strip material will be a matter of convenience, depending upon the size of the test sample involved, the **assay** protocol, the means for detecting and measuring the signal, and the like. For example, the dimensions may be chosen to. . .

SUMM As discussed above, in a binding **assay** the detection site is typically formed by directly or indirectly attaching a charged polymer to the porous material at a. . . reagent to the microparticles encompasses both covalent and non-covalent means, that is adhered, absorbed or adsorbed. It is preferred that ion-**capture** reagents be attached to the microparticles by covalent means.

SUMM . . . of the porous material will not adversely affect the performance of the device. As a result, one particularly preferred binding **assay** device uses latex particles, having **capture** reagent attached thereto, distributed in a glass fiber porous material. The distribution of the microparticles or other reagents onto or. . .

SUMM The ion-**capture** reagent, signal producing component or reagent-coated microparticles can be deposited singly or in various combinations on or in the porous. . .

SUMM Alternatively, the reagent can be distributed over the entire porous material in a substantially uniform manner to form a **capture** site or detection site that substantially includes the entire porous material. In this instance, the extent of signal production along. .

.

SUMM . . . of the porous material, wherein the reagent within each stripe is directed to a different analyte, thereby forming a multi-analyte **assay** device. As an addition to those devices in which the length or distance of analyte travel is measured, a scale. . .

SUMM . . . can be distributed more lightly at one end of the porous material than at the other. In a competitive binding **assay**, this deposition of **capture** reagent in a gradient fashion provides for greater sensitivity at the end of the porous material having the lighter distribution, because of the more rapid displacement

of the indicator reagent from the **capture** reagent binding sites by the analyte.

SUMM In alternative embodiments, the appropriate **capture** and signal producing reagents can be distributed in any pattern convenient for detection including, but not limited to, numerals, letters, dots and symbols such as ".+-.", "%" or the like which display the detectable signal upon completion of the **assay**. Reaction matrices can optionally be prepared with the **assay** reagents incorporated into the material in an overlapping design, such that the reaction of one reagent completes one portion of. . . portion of the cross. Alternatively, one portion of the design may be visible or detectable prior to performance of the **assay**, with a single reaction completing the overall design. The completion of the vertical portion alone would typically indicate a negative **assay** result, whereas completion of both portions of the detectable design would indicate a positive **assay** result. Any pattern or design may be used, however, wherein the partial formation of the design indicates other than a positive **assay** result and the complete formation of the design indicates a positive **assay** result. Such methods and devices are described in U.S. Pat. No. 4,916,056 the disclosure of which is hereby incorporated by. . .

SUMM . . . are spaced from about the proximal end of the porous material to about the distal end, thereby creating a ladder-like **capture** situs configuration. As with the narrow-stripe configuration, the bars and the intervening spaces serve to sharpen the image of the. . .

end

or low end of its concentration range. Another variation of the

parallel

bar configuration involves the use of multiple **capture** or reaction reagents wherein the reagents within the **capture** and detection sites are directed to a different analyte, thereby forming a multi-analyte **assay** device.

SUMM . . . solid phase will be a matter of convenience and will depend upon the size of the test sample involved, the **assay** protocol and the means for detecting and measuring the signal. For example, the dimensions may be chosen to regulate the. . .

SUMM Predetermined amounts of **assay** reagents can be incorporated within the device, thereby reducing or avoiding the need for additional manipulation by the user. Thus,. . . immobilized by being covalently bound to insoluble microparticles which have been deposited in and/or

on

the teststrip. More than one **assay** reagent may be present in any given reagent zone or site on the device so long as the reagents

do.

. .

SUMM The various signal display formats or patterns described above can also incorporate **assay** controls to confirm the efficacy of the **assay** reagents, the completion of the **assay** or the proper performance of the **assay**. Such controls are well-known to those skilled-in-the-art. It is also within the scope of this invention to have a reagent, at the distal end of the teststrip device, which indicates the completion of the **assay** (i.e., an end of **assay** indicator to signal that the test sample has completed its migration through the device). For example, the completion of the **assay** may be shown by a change of color at the control site upon contact with the test solution, wicking solution. . . or a signal producing component. Reagents which would change color upon contact

with

an aqueous test solution include the dehydrated **transition metal** salts, such as CuSO.sub.4, Co(NO.sub.3).sub.2, and the like. The pH indicator dyes can also be selected to respond to the. .

.

SUMM . . . to an application site or by immersing the application site in the test sample. In a sheet-like device having radial **capture** and conjugate recovery sites, the sample is applied to a central application site. Prior to contacting the sample to the solid phase,

the

sample can also be mixed with additional reagents such as the indicator reagent, **capture** reagent, buffers or wicking reagents (i.e., reagents which facilitate the transport of the test sample through the solid phase). In a further embodiment, the test sample can be applied to

one portion of the teststrip, upstream of the **capture** site, with one or more of the additional reagents being applied to yet another

portion of the teststrip upstream of. . .

SUMM In yet another embodiment, the device can include an additional absorbent material positioned downstream from or beneath the **capture** site. It will be appreciated that the absorbent material can serve to increase the amount of test sample and indicator reagent which passes through the **capture** and detection sites on the solid phase.

SUMM . . . it may be necessary to employ a wicking solution, preferably a buffered wicking solution, to facilitate the migration of the **assay** reagent(s) and test sample through the device. When an aqueous test sample is used, a wicking solution generally is not necessary but may be used to improve flow characteristics or adjust the pH of the test sample. In **immunoassays**, the wicking solution typically has a pH range from about 5.5 to about 10.5, and more preferably from about 6.5. . . test sample can be combined prior to contacting the test device, or they can be contacted to the application pad **separately**.

SUMM c. Flow-through **Assay** Devices

SUMM . . . analyte of interest. The layer is positioned such that when the

device is used in the performance of a binding **assay**, at least a portion of the test sample that contacts the first surface passes through the first surface to an. . .

SUMM The flow-through devices may also include an **assay** reagent layer or layers disposed in relation to the first layer, such that when the device is in use, sample fluid passes through the **assay** reagent layer prior to contacting the first surface. The **assay** reagent is typically resolubilized by the addition of test sample to the

reagent layer and the reagent is then available for further reaction with the analyte or other reagents housed within the **assay** device. Other embodiments may include a filter layer or a combination filter/reagent layer. Still other devices may involve a removable. . .

SUMM The novel flow-through **assay** devices of the present invention, involve a contact surface wherein a charged polymer is disposed for the nonspecific binding and immobilization of the oppositely charged **capture** reagent and complexes thereof. The device may consist of a layer or a first layer in combination with one or. . . other device

layers described above. For example, one or more pre-reaction layers may

contain the indicator reagent and or the **capture** reagent such that the analyte is allowed to contact the **assay** reagents prior to contacting the ion-**capture** surface of the flow-through device.

SUMM In either the flow-through or teststrip **assay** devices, one or more **assay** reagents, such as the indicator reagent or **capture** reagent, may be applied to the device during the performance of the **assay**. The preferred embodiments of the present invention, however, involve the incorporation of all necessary **assay** reagents into the **assay** device so that only a test sample, and in some instances a wicking solution or eluting solvent, need be applied. . .

SUMM The present invention further provides kits for carrying out binding **assays**. For example, a kit according to the present invention can comprise the **assay** device with its incorporated reagents, and can optionally include a wicking solution and/or test sample

pretreatment reagent as described above which are not incorporated in or
on the device. Other **assay** components known to those skilled-in-the-art, such as buffers, stabilizers, detergents, non-specific binding inhibitors, bacteria inhibiting agents and the like
can also be present in the **assay** device and wicking solution.

DETD The following Examples illustrate preferred ways of making the novel materials of the present invention and performing **assay** procedures using those materials. The Examples, however, are intended only to be illustrative, and are not to be construed as. . .

DETD Sandwich **Assay** for Carcinoembryonic Antigen (CEA)

DETD a. Preparation of an anti-CEA antibody-PGA **capture** reagent

DETD . . . sequence of steps describes the chemistry employed for the preparation of an antibody/polyglutamic acid (PGA) conjugate, i.e., an antibody/anionic polymer **capture** reagent.

DETD . . . with the following procedural modifications. The PDP-PGA was not reduced to the free sulfhydryl prior to the thiopropyl sepharose 6B **isolation**. Instead, the PDP-PGA was dissolved in 0.1M Na phosphate and 1 mM EDTA (pH 6.5) and stirred with thiopropyl sepharose. . .

DETD To trace the number of anionic polymer molecules attached to each **capture** reagent antibody, the TNB-protected PGA was then labeled with an ethylenediamine derivative of fluorescein. The TNB-PGA was loaded with an. . .

DETD . . . thiopropyl-fluorescein-labeled PGA was then reacted with the maleimide derived antibody to yield the antibody/PGA conjugate appropriate for a carcinoembryonic antigen ion-**capture** **immunoassay**. The maleimide-activated antibody (1.0 mg; 6.25.times.10.sup.-9 mole) in 0.1M sodium phosphate (1.0 to 2.0 ml; pH 7.0) was pH adjusted. . .

DETD The largest peak was **assayed** for protein content using Bio-Rad's Bradford **assay** with a bovine IgG standard. The peak contained 95.5 .mu.g/ml protein equating to 5.97.times.10.sup.-7 molar protein (IgG MW 160,000). By. . . this equated to 4.4 PGA molecules conjugated to each antibody. The peak fraction was frozen and subsequently used in the **assay**.

DETD As an alternative to the above **capture** reagent example, a cationic derived antibody could also be formed for use in conjunction with an anionic solid phase material.

DETD . . . conjugate of alkaline phosphatase and anti-CEA antibody fragment, which binds to a different epitope than the antibody specified
in the **capture** reagent. The alkaline phosphatase-labeled anti-CEA antibody fragment was in a buffer containing: 50 mM Tris, 50 mM
NaCl, 1.0 mM. . .

DETD d. **Immunoassay** protocol-determination of CEA

DETD The indicator reagent (70 .mu.l) was placed into a reaction well. Then, buffered **capture** reagent (20 .mu.l of anti-CEA/PGA conjugate in a buffer of 50 mM Na.sub.2 SO.sub.4, 20 mM sodium phosphate, and 300.
. . and the homogeneous immunoreaction mixture was incubated for 20 minutes at 34.5.degree. C. Four different specimens were run in the **assay**, each of which was a CEA calibrator from the Abbott Laboratories CEA enzyme **immunoassay** kit. An aliquot of each reaction mixture (100 .mu.l) was then applied to the solid phase material, followed by three. . . C. for reaction with the indicator reagent, and the resulting rate of fluorescence was measured. The dose-response results of the **assay** are shown in Table 1. The results demonstrate that as the CEA test sample concentration increased there was a corresponding increase in the formation of **capture** reagent/analyte/indicator reagent complex, and therefore, the amount of detectable label associated with the solid phase increased.

DETD TABLE 1

CEA Ion-**capture** Sandwich **Assay**
Capture reagent: anti-CEA antibody-PGA conjugate
Indicator reagent: alkaline phosphatase-labeled anti-CEA antibody fragment

CEA (ng/ml)	Rate (counts/sec/sec)
0	37
4	170
30	931
80	2398

DETD Competitive Inhibition **Assay** of Mouse Immunoglobulin
DETD a. Preparation of an IgG-PGA **capture** reagent
DETD c. Binding of the indicator reagent to the **capture** reagent
DETD . . . diluted in Tris-buffered saline containing 1% fish gelatin [25 mM Tris (hydroxymethyl) aminomethane and 100 mM NaCl, pH 7.5]. The **capture** reagent of PGA/mouse monoclonal antibody conjugate (Pool I of Table 2) was similarly treated. Two hundred microliters of each reagent. . .
DETD TABLE 3

Dose response of **capture** reagent/indicator reagent binding

PGA/antibody* (.mu.g/ml)	Rate of fluorescence (counts/sec/sec)
10	1559
1	816
0.1	179
0.01	70
0	36

*The initial concentrations of PGAcoupled-antibody before mixing. . .
DETD TABLE 4

Dose response of indicator reagent/**capture** reagent* binding

Indicator reagent titer**	Rate of fluorescence (counts/sec/sec)
10.sup.2	5062
10.sup.3	796
10.sup.4	93
10.sup.5	10
10.sup.6	5

*The initial concentration of PGAcoupled-antibody before mixing. . .
DETD d. Competitive inhibition **assay** for mouse IgG
DETD The **capture** reagent and indicator reagent were prepared as described above. All of the reagents were diluted in Tris-buffered saline containing 1% fish gelatin. The indicator reagent was diluted 1000-fold from the stock solution, and the **capture** reagent was diluted to ten .mu.g/ml. In a series of test tubes, 150 .mu.l each of appropriately diluted indicator reagent, **capture** reagent, and mouse monoclonal antibody were mixed. The mixtures were incubated at 37.degree. C. for 30 minutes. Aliquots of the. . . at 32.7.degree. C., and the resulting rate of fluorescence was measured. The results of this example illustrating a competitive inhibition **assay** for mouse IgG are shown in Table 5. The results demonstrate that as the mouse monoclonal antibody concentration increased there was a corresponding decrease in the formation of **capture** reagent/indicator reagent complex, and therefore, the amount of detectable label associated with the solid phase decreased.
DETD TABLE 5

Inhibition of indicator reagent binding due to mouse monoclonal antibody
Capture reagent: PGA/mouse monoclonal IgG conjugate

Indicator reagent: alkaline phosphatase-sheep anti-mouse immunoglobulin conjugate
Mouse IgG (.mu.g/ml)
Rate of fluorescence (counts/sec/sec)

---

0 110
3.3 .times. 10.sup.-3
106
3.3. . .
DETD Sandwich **Assay** for Human Chorionic Gonadotropin (hCG)
DETD a. Preparation of the **capture** reagent
DETD A highly negatively charged albumin derivative was prepared and coupled to anti-hCG antibodies to form the **capture** reagent according to the following procedures.
DETD . . . consisted of an alkaline phosphatase-goat anti-hCG antibody conjugate (prepared by coupling anti-hCG antibody to periodate activated
alkaline phosphatase) in an **assay** buffer containing 25 mM Tris (hydroxymethyl) aminomethane, 100 mM NaCl, 1 mM MgCl.sub.2, 0.1 mM ZnCl.sub.2, 0.07% NaN.sub.3, and 1%. . .
DETD c. Sandwich **immunoassay** protocol for hCG
DETD The ion-**capture immunoassay** protocol included the use of a solid phase prepared substantially in accordance with the method described in Example 2, the indicator reagent (alkaline phosphatase-goat anti-hCG antibody conjugate), one of two different **capture** reagents (goat anti-hCG Fab'-Sp-SUC.sub.65 -RSA and goat anti-hCG IgG-Sp-SUC.sub.65 -RSA) as prepared in Example 3.a. above, and a purified hCG standard solution. All reagents were appropriately diluted (as determined by a titer curve) in the **assay** buffer. Equal volumes (750 .mu.l) of the indicator reagent and hCG sample solution were placed in a series of test. . . After incubation at 37.degree. C. for 30 minutes, a 125 .mu.l aliquot of each incubated mixture was mixed in a **separate** tube with an equal volume of a **capture** reagent. The resulting mixtures were incubated for 30 minutes. The **assay** mixture (75 .mu.l) was then added to each solid phase material. The solid phase materials were then washed three times. . . The results demonstrate that as the hCG test sample concentration increased there was a corresponding increase in the formation of **capture** reagent/analyte/indicator reagent complex, and therefore, the amount of detectable label associated with the solid phase increased.
DETD TABLE 6

---

hCG Ion-**capture** Sandwich **Assay** Comparing Different **Capture**
Reagents
Indicator reagent: hCG-specific goat IgG-alkaline phosphatase
Rate of fluorescence (counts/sec/sec)
hCG-specific **capture** reagents
hCG (mlU/ml)
Goat IgG-Sp-SUC.sub.65 -RSA
Goat Fab'-Sp-SUC.sub.65 -RSA

0	63	64
12.5	96	110
25	121	134
50	146	166
100	182	212

DETD Indirect Sandwich Ion-**capture Immunoassay** for hCG
DETD The indirect ion-**capture immunoassay** included the use of a solid phase prepared substantially as described in Example 2 above, an indicator reagent of alkaline phosphatase-sheep anti-mouse
IgG
conjugate (Jackson ImmunoResearch Laboratories, Inc.), a **capture** reagent of goat anti-hCG F(ab').sub.2 -Sp-SUC.sub.65 -RSA as prepared
in

Example 3, an ancillary specific binding member of mouse monoclonal. . . binding member was used to bind with the analyte and the indicator reagent. All reagents were appropriately diluted in the **assay** buffer. Equal volumes (150 .mu.l) of the indicator reagent, hCG sample solution, and ancillary specific binding member were placed in a series of test tubes. After incubation at 37.degree. C. for five minutes, a 150 .mu.l portion of **capture** reagent was added to each tube. The resulting mixtures were incubated for five minutes. The **assay** mixture (200 .mu.l) was then added to each prepared solid phase material. The solid phase materials were then washed with. . . as described in Example 3. above. The resulting rate of fluorescence was measured at 32.7.degree. C. The results of the **assay** are summarized in Table 7. The results demonstrate that as the hCG test sample concentration increased there was a corresponding increase in the formation of **capture** reagent/analyte/ancillary specific binding member/indicator reagent complex, and therefore, the amount of detectable label associated with the solid phase increased.

DETD TABLE 7

Ion-**capture** Indirect Sandwich **Assay** for hCG
Capture reagent: goat anti-hCG F(ab').sub.2 -Sp-SUC.sub.65 -RSA
Indicator reagent: sheep anti-mouse IgG-alkaline phosphatase
Ancillary specific binding member: mouse monoclonal anti-hCG antibody
hCG (mlU/ml)
Rate of. . .

DETD Indirect Sandwich Ion-**capture Immunoassay** for hCG Using Two Ancillary Specific Binding Members

DETD The ion-**capture immunoassay** protocol included the use of a solid phase prepared substantially in accordance with the method described in Example 2, an. . . standard solution. Additionally, the protocol used a second ancillary specific binding member of affinity purified goat anti-hCG antibodies and a **capture** reagent of rabbit anti-goat IgG-Sp-SUC.sub.65 -RSA. The **capture** reagent was prepared by coupling affinity purified rabbit anti-goat IgG (Cappel; Cochranville, Pa., 19330) to Sp-SUC.sub.65 -RSA according to the procedure described in Example 3 above. All reagents were appropriately diluted in the **assay** buffer. Equal volumes (100 .mu.l) of the indicator reagent, hCG sample solution, and first ancillary specific binding member were placed. . . binding member (100 .mu.l) was added and the incubation was continued (at 37.degree. C. for an additional five minutes). Finally, **capture** reagent (100 .mu.l) was added to each tube. The resulting mixtures were incubated for five minutes. The **assay** mixture (200 .mu.l) was then added to each prepared solid phase material. The solid phase materials were then washed with. . . measured for the rate of fluorescence in the same manner as described in Example 3, above. The results of the **assay** are summarized in Table 8. The results demonstrate that as the hCG test sample concentration increased there was a corresponding increase in the formation of **capture** reagent/ancillary specific binding member/analyte/ancillary specific binding member/indicator reagent complex, and therefore, the amount of detectable label associated with the solid. . .

DETD TABLE 8

Ion-**capture** Indirect Sandwich **Assay** for hCG
Capture reagent: rabbit anti-goat IgG-Sp-SUC.sub.65 -RSA
Indicator reagent: sheep anti-mouse IgG-alkaline phosphatase
Ancillary specific binding member: mouse monoclonal anti-hCG antibody
Ancillary specific binding member: goat. . .

DETD Ion-**capture Immunoassay** for Anti-progesterone Antibody

DETD a. Preparation of PGA-labeled goat anti-mouse **capture** reagent

DETD . . . .mu.g; 1.25.times.10.sup.-9 mole; Sigma; in 40 .mu.l of 0.1M sodium phosphate at pH 7) to form the PGA-labeled goat anti-mouse **capture** reagent. After stirring at room temperature for two days, 0.1M Tris (20 .mu.l; pH 7.4) was added and the resulting. . .

DETD b. **Immunoassay** for anti-progesterone antibody

DETD The anti-progesterone antibody ion-**capture immunoassay** included the use of solid phase materials coated with a polymeric quaternary compound as described in Example 1. A 60. . . 1 mM MgCl.sub.2, 0.1 mM ZnCl.sub.2, and 1% BSA). After incubating the mixture at 34.5.degree. C. for ten minutes, the **capture** reagent was

L24 ANSWER 4 OF 6 USPATFULL
ACCESSION NUMBER: 1998:17226 USPATFULL
TITLE: Method and apparatus for desorption and ionization of analytes
INVENTOR(S): Hutchens, T. William, Davis, CA, United States
Yip, Tai-Tung, Davis, CA, United States
PATENT ASSIGNEE(S): Baylor College of Medicine, Houston, TX, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5719060	19980217
APPLICATION INFO.:	US 1995-483357	19950607 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1993-68896, filed on 28 May 1993
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Alexander, Lyle A.
LEGAL REPRESENTATIVE: Fulbright & Jaworski L.L.P.
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 44 Drawing Figure(s); 42 Drawing Page(s)
LINE COUNT: 2469
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates generally to methods and apparatus for desorption and ionization of analytes for the purpose of subsequent scientific analysis by such methods, for example, as mass spectrometry or biosensors. More specifically, this invention relates to the field of mass spectrometry, especially to the type of matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry used to analyze macromolecules, such as proteins or biomolecules. Most specifically, this invention relates to the sample probe geometry, sample probe composition, and sample probe surface chemistries that enable the selective capture and desorption of analytes, including intact macromolecules, directly from the probe surface into the gas (vapor) phase without added chemical matrix.

SUMM . . . costly, particularly because SDS polyacrylamide gel electrophoresis is an adequate substitute in some instances where MALDI would be applied (e.g., **separation** of crude biological fluids). In addition, MALDI has had little exposure in biological and clinical journals.

DETD . . . strong or "permanent" bonds resulting from true electron sharing), coordinate covalent bonds (e.g., between electron donor groups in proteins and **transition metal** ions such as copper or iron), and hydrophobic interactions (such as between two noncharged groups).

DETD . . . atoms in biomolecules (e.g, N, S, O) "donate" or share electrons with electron poor groups (e.g., Cu ions and other **transition metal** ions).

DETD . . . of probe elements (i.e., sample presenting means) with Surfaces Enhanced for Laser Desorption/Ionization (SELDI), within which there are three (3) **separate** subcategories. Surfaces Enhanced for Neat Desorption (SEND) where the probe element surfaces (i.e., sample presenting means) are designed to contain. . .

DETD . . . may be deliberately cut or fragmented by chemical and/or enzymatic means so that many of the resulting fragments are now

separate and distinct analytes, each one still attached (tethered) to the probe surface by one or more photolabile bonds, to be.

. .

DETD . . . said analyte molecules. In a further embodiment, said analyte molecules are biomolecules and said affinity reagent is adapted to selectively **isolate** said biomolecules from an undifferentiated biological sample. In a preferred embodiment, said matrix materials are in the weakly acidic to. . .

DETD . . . preferred embodiment presents analyte molecules are biomolecules and said affinity capture device or photolabile attachment molecule is adapted to selectively **isolate** said biomolecules from an undifferentiated biological sample.

DETD . . . is released in a light dependent manner; or, where said analyte molecules are biomolecules, said PAM is adapted to selectively **isolate** said biomolecules from an undifferentiated biological sample. In another preferred embodiment, said matrix materials are in the weakly acidic to. . .

DETD These surfaces can be derivatized (at varying densities) to bind by chemical bonds (covalent or noncovalent) affinity adsorption reagents (affinity **capture** devices), energy absorbing molecules (bound "matrix" molecules) or photolabile attachment molecules. The geometry of the sample presenting surface is varied. . . thickness, etc.) to suit the need (e.g., insertion into a living organism through spaces of predetermined sizes) of the experiment (**assay**).

DETD (Surface Enhanced Affinity **Capture**, SEAC)

DETD This example describes the use of existing and new solid phase affinity reagents designed for the (1) **capture** (adsorption) of one or more analytes, (2) the preparation of these **captured** analytes (e.g., washing with water or other buffered or nonbuffered solutions to remove contaminants such as salts, and multiple cycles. . . polar organic solvent, detergent-dissolving solvent, dilute acid, dilute base or urea), and (3) most importantly, the direct transfer of these **captured** and prepared analytes to the probe surface for subsequent analyte desorption (for detection, quantification and/or mass analysis). Affinity **capture** devices are immobilized on a variety of materials, including electrically insulating materials (porous and nonporous), flexible or nonrigid materials, optically. . . sample surface, for selective adsorption/presentation of sample for mass analysis are (1) stainless steel (or other metal) with a synthetic **polymer** coating (e.g., **cross-linked** dextran or agarose, nylon, polyethylene, polystyrene) suitable for covalent attachment of specific biomolecules or other nonbiological affinity reagents, (2) glass. . .

DETD I. Surface immobilized metal ion as the affinity **capture** device

DETD . . . IDA-Cu(II) at pH 7.0 (20 mM sodium phosphate, 0.5M sodium chloride) at 23.degree. C. for 10 min. The gel is **separated** from the remaining peptide solution by centrifugation and is then washed with 200 .mu.l sodium phosphate, sodium chloride buffer, pH. . .

DETD . . . IDA-Cu(II) at pH 7.0 (20 mM sodium phosphate, 0.5M sodium chloride) at 23.degree. C. for 10 min. The gel is **separated** from the solution by centrifugation and then washed with 500 .mu.l of buffer two times and 500 .mu.l of water. . .

DETD II. Surface immobilized antibody as the affinity **capture** device

DETD . . . affinity-adsorbed on surface immobilized antibody (if the analyte signal is unambiguously identified in a mixture of primary antibody-analyte complex, any **capture** device, e.g., surface

immobilized secondary antibody, Protein A, Protein G, Streptavidin, of the primary antibodies is used in this method. . . via specific molecular recognition events where one of the analytes is detected through its association with the primary target of **capture**; and c) the use of magnetic surface as efficient **capture** device.

DETD . . . device on a flat surface (a two-dimensional configuration) of a flexible probe element. This SEAC device may be used to **isolate** **target** analyte materials from undifferentiated biological samples such as blood, tears, urine, saliva, gastrointestinal fluids, spinal fluid, amniotic fluid, bone marrow,. . .

DETD . . . to the analyte. One way of doing this is by the combination of enzyme catalysis and the streptavidin-biotin system. After **capturing** minute quantities of lactoferrin on a nylon probe element as described in Example 3.II.2. biotinylated anti-lactoferrin antibody or biotinylated single-stranded. . . of amplification comes from the enzyme catalysis where the enzyme can achieve a turnover number of 10.sup.2 to 10.sup.3 min.sup.-1. **Assay** of alkaline phosphatase enzyme activity can easily be accomplished by using a low molecular weight phosphorylated substrate such as ATP,. . .

DETD . . . improvement of detection at the present moment is achieved by the amplification based on the polymerase chain reaction principle. After **capturing** minute quantities of lactoferrin on a nylon probe element as described in Example 3.II.2. biotinylated anti-lactoferrin antibody or biotinylated single-stranded. . .

DETD . . . spectrometry in the presence of sinapinic acid. Then the semipure preparation of human FSH (Chemicon) is digested with trypsin and **separate** aliquots (7 ul) are reacted with the mobilized antibodies (10 ul of 1:1 gel suspension) in phosphate-buffered saline at 4.degree.. . .

DETD III. Surface immobilized nucleic acid as the affinity **capture** device

DETD . . . acid are suspended in water and the pH is adjusted to 6.6 with dilute sodium hydroxide. Tentacle DEAE Fractogel (EM **Separations** , Gibbstown, N.J.) is washed with 20 mM HEPES, pH 6.0 and suction dried.

The energy absorbing molecules solution is mixed. . .

L24 ANSWER 5 OF 6 USPATFULL

ACCESSION NUMBER:	95:92727 USPATFULL
TITLE:	Ion-**capture** **assays** using a specific binding member conjugated to carboxymethylamylose
INVENTOR(S):	Adamczyk, Janina, Gurnee, IL, United States Berry, Daniel S., Libertyville, IL, United States Jou, Yi-Her, Libertyville, IL, United States Stroupe, Stephen D., Libertyville, IL, United States
PATENT ASSIGNEE(S):	Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5459080	19951017	
APPLICATION INFO.:	US 1994-187814	19940127	(8)
DISCLAIMER DATE:	20140127		
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1991-707726, filed on 30 May 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-375029, filed on 7 Jul 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-150278, filed on 29 Jan 1988, now abandoned		
DOCUMENT TYPE:	Utility		
PRIMARY EXAMINER:	Scheiner, Toni R.		
ASSISTANT EXAMINER:	Parsons, Nancy J.		

NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
LINE COUNT: 3264
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention includes novel **assays** employing a **capture** reagent, involving a first specific binding member conjugated to a polymeric anion such as carboxymethylamylose, and a solid phase material containing a reaction site comprising a polymeric cation substance. A test sample suspected of containing the analyte of interest may be contacted with the **capture** reagent to form a charged **capture** reagent/analyte complex. The complex is then contacted to/ the oppositely charged solid phase to attract, attach, and immobilize the **capture** reagent/analyte complex. The use of carboxymethylamylose to prepare a suitably charged **capture** reagent provides a superior **capture** reagent that is capable of binding and retaining the analyte on the solid phase even in the presence of polyanionic non-specific binding blockers.

TI Ion-**capture assays** using a specific binding member conjugated to carboxymethylamylose

AB The present invention includes novel **assays** employing a **capture** reagent, involving a first specific binding member conjugated to a polymeric anion such as carboxymethylamylose, and a solid phase material. . . comprising a polymeric cation substance. A test sample suspected of containing the analyte of interest may be contacted with the **capture** reagent to form a charged **capture** reagent/analyte complex. The complex is then contacted to/ the oppositely charged solid phase to attract, attach, and immobilize the **capture** reagent/analyte complex. The use of carboxymethylamylose to prepare a suitably charged **capture** reagent provides a superior **capture** reagent that is capable of binding and retaining the analyte on the solid phase even in the presence of polyanionic. . .

SUMM This invention relates generally to the field of binding **assay** devices and methods. In particular, the present invention relates to novel devices useful in the performance of homogeneous **immunoassays**.

SUMM Various analytical procedures and devices are commonly employed in **assays** to determine the presence and/or concentration of substances of interest or clinical significance which may be present in biological liquids. . .

SUMM **Immunoassay** techniques take advantage of the mechanisms of the immune systems of higher organisms, wherein antibodies are produced in response to. . .

SUMM There are several known **immunoassay** methods using immunoreactants, wherein at least one of the immunoreactants is labeled with a detectable component so as to be. . . one labeled antibody and an unlabeled antibody bound to a solid phase support such that the complex can readily be **isolated**. In this example, the amount of labeled antibody associated with the solid phase is directly proportional to the amount of. . .

SUMM An alternative technique is the "competitive" **assay**. In one example of a competitive **assay**, the **capture** mechanism again may use an antibody attached to an insoluble solid phase, but a labeled analyte (rather than a labeled. . . immobilized antibody. Similarly, an immobilized analyte can compete with the analyte of interest for a labeled antibody. In these competitive **assays**, the quantity of **captured** labeled reagent is inversely proportional to the amount of analyte present in the sample.

SUMM Despite their great utility, there are disadvantages with such **assay** methods. First, the heterogenous reaction mixture of liquid test sample and soluble and insoluble **assay** reagents, can retard the kinetics of the reaction. In comparison to a liquid phase

reaction wherein all reagents are soluble,. . . referred to as nonspecific binding and can interfere with the detection of a positive result. Third, with conventional immobilization methods, **separate** batches of manufactured solid phase reagents can contain variable amounts of immobilized binding member.

SUMM With regard to the manufacture of solid phase devices for use in binding **assays**, there are a number of **assay** devices and procedures wherein the presence of an analyte is indicated by the analyte's binding to a labeled reagent and/or. . .

SUMM The use of porous test strips in the performance of specific binding **assays** is also well-known. In a sandwich **assay** procedure, a test sample is applied to one portion of the test strip and is allowed to migrate through the. . . a component of the fluid test sample or with the aid of an eluting or chromatographic solvent which can be **separately** added to the strip. The analyte is thereby transported into a detection zone on the test strip wherein an analyte-specific. . . aid of a labeled analyte-specific binding member which may be incorporated in the test strip or which may be applied **separately** to the strip.

SUMM . . . a solution by capillary action. Different areas or zones in the strip contain the reagents needed to perform a binding **assay** and to produce a detectable signal as the analyte is transported to or through such zones. The device is suited for chemical **assays** as well as binding **assays** which are typified by the binding reaction between an antigen and a complementary antibody.

SUMM . . . an immunosorbing zone, containing an immobilized specific binding member. The test sample is applied to the immunosorbing zone, and the **assay** result is read at the immunosorbing zone.

SUMM Alternative **separation** methods include the use of a magnetic solid phase, polymerization techniques and the formation of analyte complexes having characteristics different than the non-complexed analyte. Ullman et al. (U.S. Pat. No. 4,935,147) describe a method for **separating** charged suspended non-magnetic particles from a liquid medium by contacting the particles with charged magnetic particles and a chemical reagent.. . .

SUMM Longoria et al. (U.S. Pat. No. 4,948,726) describe an **assay** method involving the reaction of antigen and antibody molecules to form an antigen/antibody complex that uniquely exhibits an ionic charge. . . is then chosen for its unique affinity for the antigen/antibody complex. Milburn et al. (U.S. Pat. No. 4,959,303) describe an **assay** wherein antigen from a test sample and an antibody specific for the antigen are incubated under conditions sufficient for the. . .

SUMM Vandekerckhove (U.S. Pat. No. 4,839,231) describes a two-stage, protein immobilization process involving an initial **separation** or **isolation** of **target** proteins in a gel, such as a polyacrylamide electrophoresis gel, followed by the transfer of those **isolated** proteins to the surface of a coated support for immobilization. The coated support is prepared by contacting a chemically inert. . .

SUMM . . . strip field. There is a growing demand for devices that require few or no manipulative steps to perform the desired **assay**, for devices that can be used by relatively untrained personnel, and for devices that provide results which are minimally affected by variations in the manner in which the **assay** is performed. Further considerations are the ease with which the resultant detection signal may be observed as well as the. . . addition, a device manufacturing format has long been sought which will enable the production of a "generic" device, i.e., an **assay** device for which the capacity of use is defined by the reagents used in the performance of the **assay** rather than the reagents used in the manufacture of the device.

SUMM The present invention provides novel binding **assay** methods for determining the presence or amount of an analyte in a test sample. The **assay** involves a **capture** reagent, containing a first binding member conjugated to a polymeric anion such as carboxymethylamylose, an indicator reagent containing a second. . . a solid phase material containing a reaction site made of a polymeric cation substance. The specific binding members of the **capture** reagent and indicator are chosen for the formation of a complex with the analyte in a sandwich **assay**, a competitive **assay** or an indirect **assay**, thereby forming a detectable complex in proportion to the presence or amount of the analyte in the test sample.

SUMM The solid phase is contacted with the **capture** reagent and the test sample, whereby the polymeric cation of the solid phase attracts and attaches to the polymeric anion of the **capture** reagent, thereby immobilizing the **capture** reagent and complexes thereof upon the solid phase. The solid phase may then be contacted with the indicator reagent, whereby the indicator reagent becomes bound to the immobilized **capture** reagent, or complex thereof, in proportion to the amount of analyte present in the test sample. Typically, the indicator reagent. . .

SUMM The present invention also enables the production of a generic solid phase device for use in specific binding **assays**. **Assay** procedures for many different analytes can use the same solid phase material which contains a predetermined zone of anionic or cationic **capture** polymer rather than an immobilized binding member capable of binding only a specific analyte as found in conventional flow-through and. . .

SUMM The specific binding member component of the **capture** reagent can be either a hapten or a macromolecule. The charged **capture** reagent enables homogeneous **assay** and **separation** reactions wherein the reaction complexes can be removed from the reaction mixture by contacting the mixture with an oppositely charged solid phase. Virtually any binding **assay** (sandwich **assays**, competitive **assays**, indirect **assays**, **assays** using ancillary specific binding members, inhibition **assays**, etc.) can be adapted to use the novel **capture** reagents and ion-**capture** techniques of the present invention.

SUMM The present invention provides two major advancements to the field of specific binding **assays**: a) the use of liquid phase kinetics facilitates the formation of a complex from the homogeneous mixture of analyte and **assay** reagent specific binding members, and b) the ion-**capture** technique increases the potential number of complexes that can be immobilized on a solid support. If the advantages of liquid. . .

SUMM The novel **capture** reagent of the present invention can also be used in a **separation** procedure. A liquid sample containing an analyte to be **separated** from the sample is mixed with the **capture** reagent and reacted to form a charged analyte/ **capture** reagent complex. Following the specific binding reaction, the solution is contacted to an oppositely charged solid phase which attracts, attaches to, and **separates** the newly formed complex from the liquid sample.

DETD The **assay** methods and reagents of the present invention can be used in a variety of **immunoassay** formats. The present invention, however, is not limited to immunoreactive **assays**. Any **assays** using specific binding reactions between the analyte and **assay** reagents can be performed.

DETD . . . specific binding pairs are exemplified by the following: biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences (including probe and **capture** nucleic acid sequences used in DNA hybridization **assays** to detect a target nucleic acid sequence), complementary peptide sequences (including those formed

by recombinant methods), effector and receptor molecules,. . .

DETD . . . pretreated prior to use, such as preparing plasma from blood, diluting viscous liquids, etc. Methods of pretreatment can also involve **separation**, filtration, distillation, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples such as. . .

DETD The term "analyte", as used herein, refers to the substance to be detected in or **separated** from the test sample by means of the present invention. The analyte can be any substance for which there exists. . .

DETD The term "signal producing component", as used herein, refers to any substance capable of reacting with the analyte or another **assay** reagent to produce a reaction product or signal that indicates the presence or amount of the analyte and that is detectable by visual or instrumental means. "Signal production system", as used herein, refers to the group of **assay** reagents that are used to produce the desired reaction product or signal. For example, one or more signal producing components. . .

DETD . . . the amount of an analyte in the test sample. Generally, the indicator reagent is detected or measured after it is **captured** on the solid phase material, but the unbound indicator reagent can also be measured to determine the result of an **assay**.

DETD The specific binding member of the indicator reagent is capable of binding either to the analyte as in a sandwich **assay**, to the **capture** reagent as in a competitive **assay**, or to an ancillary specific binding member to complete a detectable complex. The label, as described above, enables the indicator. . . reagent enables the indirect binding of the label to the analyte, to an ancillary specific binding member or to the **capture** reagent. The selection of a particular label is not critical, but the label will be capable of generating a detectable. . .

DETD As mentioned above, the label can become attached to the specific binding member during the course of the **assay**. For example, a biotinylated anti-analyte antibody may be reacted with a labeled streptavidin molecule. Any suitable combination of binding members. . .

DETD The term "**capture** reagent", as used herein, refers to an unlabeled specific binding member which is attached to a charged substance. The attachment. . . attachment to the charged substance does not interfere with the binding member's binding site. The binding member component of the **capture** reagent is specific either for the analyte as in a sandwich **assay**, for the indicator reagent or analyte as in a competitive **assay**, or for an ancillary specific binding member, which itself is specific for the analyte.

DETD The charged substance component of the **capture** reagent can include anionic and cationic monomers or polymers. For example, anionic polymers include polyglutamic acid (PGA), anionic protein or. . . member can be joined to more than one charged monomer or polymer to increase the net charge associated with the **capture** reagent.

DETD The novel **capture** reagents of the present invention are used to facilitate the observation of the detectable signal by substantially **separating** the analyte and/or the indicator reagent from other **assay** reagents and the remaining test sample components. In its most advantageous use, the **capture** reagent is reacted with the test sample and **assay** reagents in a homogeneous reaction mixture. Following the formation of the desired specific binding member complexes, the complexes involving a **capture** reagent are removed from the homogeneous reaction mixture by contacting the homogeneous reaction mixture to a solid phase that is oppositely charged with respect to the charge of the **capture** reagent.

DETD In one embodiment of the present invention, a negatively charged **capture** reagent can be prepared by conjugating the selected specific binding member to one or more activated polymeric anionic molecules and. . .

DETD Typically, the negatively charged **capture** reagents of the following Examples were formed by reacting the desired specific binding member with an activated PGA molecule having. . .

DETD . . . used to "activate" a specific binding member or polymeric anionic molecule, i.e., to prepare the specific binding member or the **polymeric** anionic molecule for chemical **cross-linking**. Activating agents also include thiol introducing agents such as the thiolanes (such as 2-iminothiolane), succinimidyl mercaptoacetates (such as N-succinimidyI-S-acetylmercaptoacetate), and. . .

DETD . . . preferred agents for use with the particular polymeric anionic molecule and specific binding member to be used in the diagnostic **assay**. Therefore, it will be appreciated by those skilled-in-the-art that the coupling agent or activating agent used in a given **assay** will generally be determined empirically.

DETD . . . a sulfur stabilizer and R" is an aliphatic or aryl group. An example of the preparation of a negatively charged **capture** reagent involves the reaction of a specific binding member (SBM) having an amino group and an activated PGA having an. . .

DETD In yet another embodiment of the present invention, a preferred anionic polymer for use in the **capture** reagent is carboxymethylamylose (CMA) due to its particular performance in various **immunoassay** configurations. The improved performance of **capture** reagents containing CMA can be attributed to the higher avidity of the CMA **capture** reagent for the cationic solid phase. This attribute is particularly advantageous in a two step sandwich **assay** format wherein a polyanion is used to block nonspecific binding of the indicator reagent to the cationic solid phase.

DETD . . . specific binding member", as used herein, refers to any member of a specific binding pair which is used in the **assay** in addition to the specific binding members of the **capture** reagent and the indicator reagent. For example, in an **assay** an ancillary specific binding member may bind the analyte to a second specific binding member to which the analyte itself could not attach, or as in an inhibition **assay** the ancillary specific binding member may be a reference binding member. One or more ancillary specific binding members can be used in an **assay**.

DETD . . . insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic charge and ability to attract the **capture** reagent, e.g., methylated wool, nylons, and special glasses having a positive charge. Alternatively, the solid phase can be pretreated with and retain a charged substance that is oppositely charged with respect to the charged substance of the **capture** reagent. For example, an anionic substance can be bound to a specific binding member to form the **capture** reagent, and a cationic substance can be applied to and retained by the solid phase, or vice versa.

DETD . . . about 2% (exclusive of counter ion) are particularly advantageous in preparing a solid phase that will undergo washing during the **assay** process. The use of such a polycationic substance to prepare a suitably charged solid phase resulted in a solid phase. . . could be subjected to a greater degree of manipulation without losing the capability to attract and retain the oppositely charged **capture** reagent. It was determined that polycationic substances having a nitrogen content above about 5% (exclusive of counter ion) were more. . .

DETD An **assay** device based on the ion-**capture** technique can have many configurations, several of which are dependent upon the material chosen as the solid phase. In various. . .

DETD The novel ion-**capture** devices of the present invention involve a solid phase made of any suitable porous material. By "porous" is meant

that. . . phase materials. For example, the solid phase can include a
fiberglass, cellulose, or nylon pad for use in a flow-through **assay** device having one or more layers containing one or more of the **assay** reagents; a dipstick for a dip and read **assay**; a test strip for wicking or capillary action (e.g., paper, nitrocellulose, polyethylene) techniques; or other porous or open
pore materials. . .

DETD . . . cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon); porous gels such as silica gel, agarose, dextran, and gelatin; **polymeric** films such as **polyacrylamide**; and the like. The solid phase should have reasonable strength or strength can be provided by means of a support,. . .

DETD Preferred solid phase materials for flow-through **assay** devices include filter paper such as a porous fiberglass material or other fiber
matrix materials as well as isotropically porous. . . the material used will be a matter of choice, largely based upon the properties of the sample or analyte being **assayed**, e.g., the fluidity of the test sample.

DETD Typically, the novel test strip and flow-through devices employing the ion-**capture** principles of the present invention are characterized by having the analyte, test sample and/or eluting solvent migrate through the device. . .

DETD Uses for Ion-**Capture** Reagents

DETD In accordance with the disclosure of the present invention, a sandwich **assay** can be performed wherein the **capture** reagent involves an analyte-specific binding member which has been bound to a charged substance such as an anionic polymer. The **capture** reagent is contacted with a test sample, suspected of containing the analyte, and an indicator reagent comprising a labeled analyte-specific.
. .

DETD In the exemplary sandwich **assay**, a binding reaction results in the formation of a **capture** reagent/analyte/indicator reagent complex. The resultant complex is then removed from the excess **assay** reagents and test sample of the homogenous reaction mixture by means of a solid phase that is either inherently oppositely charged with respect to the **capture** reagent or that retains an oppositely charged substance, for example a cationic polymer. In the ion-**capture assays**, the oppositely charged solid phase attracts and attaches to the **capture** reagent/analyte/indicator reagent complex through the interaction of the
anionic and cationic polymers. The complex retained on the solid phase is. . . solid phase is proportional to the amount of analyte in the sample. The only major limitation inherent in the sandwich **assay** is the requirement for the analyte to have a sufficient size and appropriately orientated epitopes to permit the binding of at least two specific binding members. Other sandwich **assays** may involve one or more ancillary specific binding members to bind the analyte to the indicator reagent and/or **capture** reagent.

DETD The present invention also can be used to conduct a competitive **assay**. In an exemplary competitive **assay**, the soluble **capture** reagent again includes a specific binding member which has been attached to a charged substance, such as an anionic polymer. The **capture** reagent is contacted, either sequentially or simultaneously, with the test sample and an indicator reagent that includes a second binding member which has been labeled with a signal generating compound. Either the **capture** reagent and analyte can compete in binding to the indicator reagent (e.g., the **capture** reagent and analyte are antigens competing for a labeled antibody), or the indicator reagent and analyte can compete in binding to the **capture** reagent (e.g., the indicator reagent is a labeled antigen which competes with the antigen analyte for binding to

the antibody component of the **capture** reagent). A competitive binding or displacement reaction occurs in the homogeneous mixture and results in the formation of **capture** reagent/analyte complexes and **capture** reagent/indicator reagent complexes.

DETD The resultant complexes are removed from the excess **assay** reagents and test sample by contacting the reaction mixture with the oppositely charged solid phase. The **capture** reagent complexes are retained on the solid phase through the interaction of the oppositely charged polymers. The complexes retained on the solid phase can be detected via the label of the indicator reagent. In the competitive **assay**, the amount of label that becomes associated with the solid phase is inversely proportional to the amount of analyte in the sample. Thus, a positive test sample will generate a negative signal. The competitive **assay** is advantageously used to determine the presence of small molecule analytes, such as small peptides or haptens, which have a single epitope with which to bind a specific binding partner. Other competitive **assays** may involve one or more ancillary specific binding members to bind the analyte to the indicator reagent and/or **capture** reagent.

DETD For example, in an **assay** for theophylline, an anti-theophylline antibody (either monoclonal or polycional) can be conjugated with an anionic polymer to form a soluble **capture** reagent, and a competition for binding to that antibody can be established between labeled theophylline (i.e., indicator reagent) and the. . . to a solid phase which retains a cationic polymer coating. The attraction between the oppositely charged ionic species of the **capture** reagent and the solid phase serves to **separate** the immunocomplex from the reaction mixture. The signal from the indicator reagent can then be detected. In this example, increased. . .

DETD In addition, the present invention can be used in an inhibition **assay**, such as the measurement of an antibody by inhibiting the detection of a reference antigen. For example, the **capture** reagent can include an antibody/anionic polymer conjugate and the indicator reagent can be a labeled antibody. The test sample, suspected of containing an antibody analyte, is mixed with a reference antigen with which the **capture** reagent and indicator reagent can form a detectable sandwich complex that can be immobilized upon the solid phase by the ion-**capture** reaction. The degree of inhibition of antigen uptake by the **capture** reagent is proportional to the amount of antibody analyte in the test sample, thus, as the concentration of the antibody. . .

DETD In general, once complex formation occurs between the analyte and the **assay** reagents, the oppositely charged solid phase is used as a **separation** mechanism: the homogeneous reaction mixture is contacted with the solid phase, and the newly formed binding complexes are retained on the solid phase through the interaction of the opposite charges of the solid phase and the **capture** reagent. If the user is not concerned with liquid phase kinetics, the **capture** reagent can be pre-immobilized on the solid phase to form a **capture** site.

DETD The present invention can also be used for **separating** a substance from a liquid sample. For example, the **capture** reagent and solid phase can be used without an indicator reagent for the sole purpose of **separating** an analyte from a test sample. Furthermore, the **capture** reagent can be contacted with a soluble second charged substance which is oppositely charged with respect to the **capture** reagent. The second charged substance is not retained on the solid phase prior to contacting the sample to the solid phase material, but it attracts and attaches to the **capture** reagent such that the resultant **assay** complexes are retained on an oppositely charged solid phase.

DETD When the complex of charged **capture** reagent and analyte (and/or indicator reagent) is contacted to the oppositely charged solid

phase, the ionic attraction of the oppositely charged species governs the efficiency of the **separation** of the complex from the reaction mixture. The ionic attraction can be selected to provide a greater attraction than the immunological attraction of antibody for antigen, particularly when multiple polycationic and polyanionic species are included in the **capture** reagent and oppositely charged solid phase. A further advantage is that the "ion-**capture**" technique minimizes the nonspecific adsorption of interfering substances onto the solid phase, thereby offering improved accuracy of analysis. The ion-**capture** technique thereby enables the performance of an **assay** having a highly specific **separation** method, minimal nonspecific binding, and high sensitivity.

DETD . . . to noise ratio. It was unexpectedly discovered that the nonspecific binding blocker could be a free polyanion even when the **capture** reagent used in the **assay** involved a polyanionic substance conjugated to a specific binding member. It would have been expected that the presence of a free or unbound polyanion would prevent, or at least reduce, the immobilization of the **capture** reagent on the solid phase. It was found, however, that the nonspecific blocker was more effective in inhibiting the direct, nonspecific binding of indicator reagent to the solid phase than it was in reducing the attachment of the polyanionic **capture** reagent to the polycationic solid phase. Suitable nonspecific binding blockers include, but are not limited to, dextran sulfate, heparin, carboxymethyl. . .

DETD . . . nonspecific binding blocker added to the indicator reagent could be greater than the amount of polyanionic substance contained in the **capture** reagent. It was found that free polyanionic nonspecific binding blocker could be added to the indicator reagent in amounts 40,000 times the amount of polyanionic substance used in the **capture** reagent. Generally, the preferred amount of polyanionic blocker added to the indicator reagent is 50 to 14,000 times the amount of polyanionic substance used in the **capture** reagent. For two step sandwich **assays**, the preferred amount of polyanionic blocker added to the indicator reagent is 1000 to 2000 times that contained in the **capture** reagent.

DETD An appropriate range of use can be determined for each analyte of interest. For example, in an **assay** to detect thyroid stimulating hormone (TSH) wherein dextran sulfate was added to the indicator reagent as a free polyanionic nonspecific binding blocker, suitable amounts of free polyanion ranged from 233 to 19,000 times that of the **capture** reagent, or about 0.1-8% dextran sulfate. As illustrated in the following Table, the preferred nonspecific binding blocker as well as. . .

DETD

Nonspecific Binding Blocker in the Indicator Reagent Analyte	Preferred	More Preferred
	% Dextran sulfate (MW 5,000) (blocker/**capture** reagent, w/w)	
TSH	0.1-8 (233-19,000)	0.5-2 (1,000-4,000)
T3	0.1-2 (2,000-40,000)	0.1-0.2 (2,000-4,000)
	% Carboxymethyl cellulose (MW 250,000) (blocker/**capture** reagent, w/w)	
hCG	0.01-0.25 (0.44-11)	0.025 (1.1)
HIV	0-0.2 (0-20,000)	0.05

(5,000)

DETD Moreover, it was discovered that the polyanionic nonspecific binding blocker could be added to the **assay** as a **separate** reagent, or it could be included as free polyanion in the **capture** reagent, in an ancillary binding member reagent, in a buffer reagent or in some other reagent used in the **assay**. For example, when free polyanion is included in the **capture** reagent, it can enhance the signal to noise ratio by neutralizing interfering materials which are contained either in the test sample itself or in the other **assay** reagents, or those which were introduced during the device manufacturing process. The following Table illustrates some preferred amounts of nonspecific binding blocker for different analytes of interest, wherein the free polyanion is contained in the **capture** reagent itself.

DETD

Nonspecific Binding Blocker in the **Capture** Reagent Analyte	Preferred	More Preferred
	% Dextran sulfate (MW 5,000) (blocker/**capture** reagent, w/w)	
Digoxin	0-0.004 (0-222)	0.004 (222)
T3	0.004-0.01 (66-165)	0.004 (66)

DETD Depending upon the analyte of interest and the desired **assay** configuration, the preferred nonspecific binding blocker, as well as the optimization of its concentration and whether it is included as a component of another **assay** reagent, is selected by empirical

L29 ANSWER 6 OF 12 USPATFULL
ACCESSION NUMBER: 1998:17226 USPATFULL
TITLE: Method and apparatus for desorption and ionization of analytes
INVENTOR(S): Hutchens, T. William, Davis, CA, United States
Yip, Tai-Tung, Davis, CA, United States
PATENT ASSIGNEE(S): Baylor College of Medicine, Houston, TX, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5719060	19980217
APPLICATION INFO.:	US 1995-483357	19950607 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1993-68896, filed on 28 May 1993
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Alexander, Lyle A.
LEGAL REPRESENTATIVE: Fulbright & Jaworski L.L.P.
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 44 Drawing Figure(s); 42 Drawing Page(s)
LINE COUNT: 2469
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates generally to methods and apparatus for desorption and ionization of analytes for the purpose of subsequent scientific analysis by such methods, for example, as mass spectrometry or biosensors. More specifically, this invention relates to the field of mass spectrometry, especially to the type of matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry used to analyze macromolecules, such as proteins or biomolecules. Most specifically, this invention relates to the sample probe geometry, sample probe composition, and sample probe surface chemistries that enable the selective capture and desorption of analytes, including intact macromolecules, directly from the probe surface into the gas (vapor) phase without added chemical matrix.

DETD . . . strong or "permanent" bonds resulting from true electron sharing), coordinate covalent bonds (e.g., between electron donor groups in proteins and **transition metal** ions such as copper or iron), and hydrophobic interactions (such as between two noncharged groups).

DETD . . . atoms in biomolecules (e.g, N, S, O) "donate" or share electrons with electron poor groups (e.g., Cu ions and other **transition metal** ions).

DETD . . . sample surface, for selective adsorption/presentation of sample for mass analysis are (1) stainless steel (or other metal) with a synthetic **polymer** coating (e.g., **cross-linked** dextran or agarose, nylon, polyethylene, polystyrene) suitable for covalent attachment of specific biomolecules or other nonbiological affinity reagents, (2) glass. . .

DETD . . . device on a flat surface (a two-dimensional configuration) of a flexible probe element. This SEAC device may be used to **isolate target** analyte materials from undifferentiated biological samples such as blood, tears, urine, saliva, gastrointestinal fluids, spinal fluid, amniotic fluid, bone marrow,. . .

L29 ANSWER 8 OF 12 USPATFULL
ACCESSION NUMBER: 96:70333 USPATFULL
TITLE: Methods and materials for improved high gradient magnetic separation of biological materials
INVENTOR(S): Miltenyi, Stefan, Moitzfeld 60a, D-51429 Bergisch Gladbach 1, Germany, Federal Republic of

	NUMBER	DATE
PATENT INFORMATION:	US 5543289	19960806
APPLICATION INFO.:	US 1994-252102	19940531 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-20019, filed on 17 Feb 1993, now patented, Pat. No. US 5411863 which is a continuation of Ser. No. US 1988-291177, filed on 28 Dec 1988, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Feisee, Lila
ASSISTANT EXAMINER: Wolski, Susan C.
LEGAL REPRESENTATIVE: Morrison & Foerster
NUMBER OF CLAIMS: 3
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT: 802
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Improvements in the existing procedures and materials for conduct of high gradient magnetic separation (HGMS) are disclosed. Superior superparamagnetic particles, optionally coated with a polysaccharide or other, usually organic, materials can be prepared in uniform compositions with homogeneous magnetizations. The coating can conveniently be conjugated to a specific binding moiety complementary to a biological material whose purification or separation is desired. In addition, plastic coated matrices which form superior magnetic gradient-intensifying supports are disclosed, along with improved methods and apparatus to conduct HGMS.

SUMM . . . are provided, wherein the coating can be conjugated to moieties which confer on the particle specificity for binding to the **target** material whose **isolation** is desired. A particularly preferred coating is comprised of polysaccharide. Accordingly, in another aspect, the invention is directed to a. . .

DETD . . . in the iron of the hemoglobin contained in them; ferromagnetic particles are those where the iron atoms (or other magnetic **transition metal**) are highly coupled. Superparamagnetic materials occupy the range between these extremes.

DETD . . . contains substantially less than 30% water by weight, and is coated to the matrix as a result of passive application, **cross -linking** or **polymerization** of a relatively hydrophobic polymer or copolymer. A variety of materials can be used to form impermeable membranes, as is generally understood in the art. Suitable **polymers** include polystyrenes, **polyacrylamides**, polyetherurethanes, polysulfones, fluoronated or chlorinated **polymers** such as polyvinyl chloride, polyethylenes and polypropylenes, polycarbonates and polyesters. Other polymers include polyolefins such as polybutadiene, polydichlorobutadiene, polyisoprene, polychloroprene,. . .

L29 ANSWER 11 OF 12 USPATFULL
ACCESSION NUMBER: 95:38576 USPATFULL
TITLE: Methods and materials for improved high gradient magnetic separation of biological materials
INVENTOR(S): Miltenyi, Stefan, Moitzfeld 60a, D-5060 Bergisch Gladbach 1, Cologne, Germany, Federal Republic of
PATENT ASSIGNEE(S): Miltenyi, S., Germany, Federal Republic of (non-U.S.

individual)

	NUMBER	DATE
PATENT INFORMATION:	US 5411863	19950502
APPLICATION INFO.:	US 1993-20019	19930217 (8)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1988-291177, filed on 28 Dec 1988, now abandoned	
DOCUMENT TYPE:	Utility	
PRIMARY EXAMINER:	Parr, Margaret	
ASSISTANT EXAMINER:	Marschel, Ardin H.	
LEGAL REPRESENTATIVE:	Morrison & Foerster	
NUMBER OF CLAIMS:	17	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	4 Drawing Figure(s); 2 Drawing Page(s)	
LINE COUNT:	945	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Improvements in the existing procedures and materials for conduct of high gradient magnetic separation (HGMS) are disclosed. Superior superparamagnetic particles, optionally coated with a polysaccharide or other, usually organic, materials can be prepared in uniform compositions with homogeneous magnetizations. The coating can conveniently be conjugated to a specific binding moiety complementary to a biological material whose purification or separation is desired. In addition, plastic coated matrices which form superior magnetic gradient-intensifying supports are disclosed, along with improved methods and apparatus to conduct HGMS.

SUMM . . . are provided, wherein the coating can be conjugated to moieties which confer on the particle specificity for binding to the **target** material whose **isolation** is desired. A particularly preferred coating is comprised of polysaccharide. Accordingly, in another aspect, the invention is directed to a. . .

DETD . . . in the iron of the hemoglobin contained in them; ferromagnetic particles are those where the iron atoms (or other magnetic **transition metal**) are highly coupled. Superparamagnetic materials occupy the range between these extremes.

DETD . . . contains substantially less than 30% water by weight, and is coated to the matrix as a result of passive application, **cross -linking** or **polymerization** of a relatively hydrophobic polymer or co-polymer. A variety of materials can be used to form impermeable membranes, as is generally understood in the art. Suitable **polymers** include polystyrenes, **polyacrylamides**, polyetherurethanes, polysulfones, fluoronated or chlorinated **polymers** such as polyvinyl chloride, polyethylenes and polypropylenes, polycarbonates and polyesters. Other polymers include polyolefins such as polybutadiene, polydichlorobutadiene, polyisoprene, polychloroprene,. . .

L12 ANSWER 3 OF 3 USPATFULL on STN
ACCESSION NUMBER: 2002:22108 USPATFULL
TITLE: ELECTROCHEMICAL PROBES FOR DETECTION OF MOLECULAR INTERACTIONS AND DRUG DISCOVERY
INVENTOR(S): FOWLKES, DANA M., CHAPEL HILL, NC, UNITED STATES
THORP, H. HOLDEN, CHAPEL HILL, NC, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002012943	A1	20020131
APPLICATION INFO.:	US 1998-19679	A1	19980206 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-36919P	19970206 (60)
	US 1997-59049P	19970916 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MYERS BIGEL SIBLEY & SAJOVEC, PO BOX 37428, RALEIGH, NC, 27627
NUMBER OF CLAIMS: 60
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 14 Drawing Page(s)
LINE COUNT: 2944
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to methods and apparati for performing electrochemical analyses. The invention provides an electrochemical apparatus for performing potentio metric analyses for detecting specific binding between a first member of a biological binding pair immobilized on an electrode and a second member of a biological binding pair that is electrochemically labeled, in the presence of an electrochemical mediator. Methods for using the apparatus of the invention for performing binding and competition binding assays are provided. The invention also provides methods for performing high throughput screening assays for detecting inhibition of specific binding between the members of the biological binding pair for use in drug development, biochemical analysis and protein purification assays.

SUMM [0008] Ford et al., 1968, J. Amer. Chem. Soc. 90: 1187-1194 describes synthesis of pentaamine-ruthenium complexes of aromatic **nitrogen** heterocycles.

SUMM . . . Yocum et al., 1982, Proc. Natl. Acad. Sci. USA 79: 7052-7055 describes preparation of a pentaammineruthenium derivative of horse heart **ferricytochrome** c.

SUMM [0062] **Gold** et al., 1995, Ann. Rev. Biochem. 64: 763-797 describes aptamers.

SUMM [0067] Wrighton et al., 1996, Science 273:458-463 describes small peptides **isolated** from random peptide libraries as mimetics for erythropoietin.

SUMM [0113] In an additional aspect of this embodiment of the invention, the method is used to **isolate** and identify an inhibitor of binding of the second member of the biological binding pair to the first member of. . .

SUMM . . . be determined by analysis of the extent of produced current in the presence of putative inhibitors, competitors, activators or drug **lead** candidates, wherein specific details of the performance of such comparisons will be understood by those with skill in the art. . .

SUMM [0158] In an additional aspect of this embodiment of the invention, the method is used to **isolate** and identify an inhibitor of binding of the second member of the biological binding pair to the first member of. . .

SUMM . . . be determined by analysis of the extent of produced current in the presence of putative inhibitors, competitors, activators or drug

lead candidates, wherein specific details of the performance of such comparisons will be understood by those with skill in the art. . .

SUMM [0204] In an additional aspect of this embodiment of the invention, the method is used to **isolate** and identify an inhibitor of binding of the second member of the biological binding pair to the first member of. . .

DETD . . . pair. For the purposes of this invention, the term "biological binding pair" is intended to encompass any two biologically-derived or **isolated** molecules, or any chemical species that specifically interact therewith, that specifically bind with a chemical affinity measured by a dissociation. . . of the invention are intended to encompass molecules that are naturally-occurring, synthetic, or prepared by recombinant genetic means or biochemical **isolation** and extraction means. Synthetic embodiments of a member of a biological binding pair will be understood to typically share structural. . .

DETD . . . conductive or semiconductive electrodes of the invention include metallically-impregnated glass, such as tin-doped indium oxide or fluorine-doped tin oxide glass, **gold**, carbon or **platinum**. Examples of materials useful as coatings for the first electrode of the invention include agar, agarose, dextrans and modified dextrans, **acrylamide**, pyrroles and pyrrole-**carboxylates**, polystyrene, nylon, nitrocellulose, mylar, Nafion, polyethylene, polypropylene, polypyrroles, polythiophene, and polyaniline. The coating of the first electrodes of the invention. . . electrode coating material. For example, carbodiimide crosslinking is useful when the electrodes contain oxidized mylar on metal oxide, carbon or **gold**, oxidized polystyrene on carbon or **gold**, alkanethiol-**carboxylate** self-assembled monolayers (SAMS) on **gold**, **carboxylate** SAMS on metal oxides, or electropolymerized **carboxylate**-containing monomers. Alternatively, avidin or streptavidin can be attached to the electrode by any of the above means or by passive. . .

DETD . . . member is electrochemically labeled. Electrochemical labels are defined as chemical species, typically cationic species comprising cations including ruthenium, osmium or **cobalt**, that are capable of participating in a reduction/oxidation (redox) reaction with the electrochemical mediator and the first electrode of the. . . of the apparatus. For second members of a biological binding pair comprising a peptide, inorganic complexes such as Ru.sup.2+/3+-amine complexes, **ferrocenes**, and osmium- or **cobalt** -polypyridyl complexes are attached to the peptide via histidine or cysteine residues or at the amino terminus. Redox-active organic molecules, such. . .

DETD . . . the electrochemical probes and targets of the apparati and methods of this invention can be prepared by synthetic methods, including **solid phase** peptide synthesis, biochemical **isolation** and modification techniques including partial proteolysis, and by recombinant genetic methods understood by those with skill in the art (see. . .

DETD . . . ruthenium group (RU(NH.sub.3).sub.5(OH.sub.2).sub.2.sup.2+) to histidine residues within the peptide sequence. Alternatively, electrochemical labels can be added to the amino- or **carboxyl** termini post-synthetically, or to the reactive side chain thiol groups of a cysteine residue, the hydroxyl group of a serine. . .

DETD . . . a target molecule can be obtained from combinatorial nucleic acid libraries; these molecules have been termed "aptamers" (as disclosed in **Gold** et al., 1995, Ann. Rev. Biochem. 64: 763). Such aptamers can be electrochemically-labeled with a labeling group at either the. . .

DETD . . . cutoff threshold, 5 kD. This limitation is important, since small molecular weight compounds form a large percentage of potential drug **lead** compounds. In addition, assay conditions using the methods and apparati of this invention are more permissible than the

assay conditions. . .

DETD . . . a reaction chamber comprising more than one immobilized target protein-comprising electrode, so that one or a mixture of potential drug **lead** compounds can be analyzed for binding to a variety of potential targets. Sixth, the methods and apparati of the invention. . .

DETD [0244] The apparatus of the invention also provides a **hydrophilic polymer** modified electrode containing the first member of a biological binding pair, preferably a protein and most preferably a receptor or. . . a biological binding pair, such as proteins, and electrochemical mediators are chemically linked to the polymeric support either directly through **covalent** bond formation between reactive groups or through mutually reactive chemical **linkers**. For example, the side chains of several amino acids contain nucleophilic heteroatoms that can undergo addition to epoxide functionalities in. . . via bifunctional activated electrophiles such as dicyclohexylcarbodiimide-, N-hydroxysuccinimide-, or hydroxybenzotriazole-activated dicarboxylates. Techniques for coupling electrochemical mediators include coordination of a **transition -metal** complex to nucleophilic atoms on the polymer, incorporation of a reactive group into an organic mediator or metal-complex ligand, or incorporation of **transition-metal**-binding sites along the polymer backbone. For example, coordination of polyvinylimidazole to bisbipyridinechloroosmium(II) yields a very stable polymer in which Os(II) and Os(III) interconvert at modest applied potentials. Chemical modifications of bipyridine ligands have resulted in metal complexes containing activated **carboxylate** moieties for coupling to nucleophiles and other functional groups that allow direct incorporation of complexes in the context of automated. . .

DETD . . . of electrode potential allows selective electrochemical detection of the enzyme-catalyzed reaction in the vicinity of the electrode. Also, several synthetic **transition-metal** complexes such as those of oxoruthenium(IV), oxoosmium(IV), oxomolybdenum(IV), dioxomolybdenum(VI) and dioxorhenium(VI) are capable of oxidizing or reducing a variety of. . .

DETD [0251] is labeled as follows. The electrochemical label Ru(NH.sub.3).sub.5(H.sub.2O).sup.2+ was generated by the reduction of {Ru(NH.sub.3).sub.5Cl}Cl.sub.2 over **zinc**/mercury amalgam, using conventional techniques (see Ford et al., 1968, J. Amer. Chem. Soc. 90: 1187-1194; Vogt et al., 1965, Inorg.. . .

DETD . . . 273A potentiostat/galvanostat with a single compartment voltammetric cell equipped with a modified tin-doped indium oxide (ITO) working electrode (area=0.32 cm.sup.2), **platinum** (Pt)-wire counter electrode and silver/silver chloride (Ag/AgCl) reference electrode (see Johnston et al, 1994, Inorg. Chem. 33: 6388-6390). An example. . .

DETD . . . the tumor suppressor gene, p53. Electrodes coated as described above in Example 2 with the target protein MDM2 is used **capture** a surrogate ligand having a amino acid sequence derived from the native amino acid sequence of p53:

DETD . . . electrochemical analyzer (BAS, West Lafayette, Ind.). The src SH3-hydrogel coated electrode described above, a Ag/AgCl reference electrode (BAS) and a **platinum** auxiliary electrode were immersed in a 5 mL solution of PBS containing 1% bovine serum albumin. The solution was stirred. . .

=>